

UNITED STATES PATENT
APPLICATION OF

DAVID J. CARINI
23 Huntington Ridge Road
Wallingford, CT 06492

FOR

ALPHA, ALPHA-DISUBSTITUTED BENZYLGLYCINE DERIVATIVES
AS HIV PROTEASE INHIBITORS

EXPRESS MAILING LABEL NO. EL 994356742US
DATE OF DEPOSIT November 3, 2003

I HEREBY CERTIFY THAT THE ATTACHED CORRESPONDENCE AND DOCUMENTS ARE BEING DEPOSITED WITH THE U.S. POSTAL SERVICE "EXPRESS MAIL POST OFFICE TO ADDRESSEE" SERVICE UNDER 37 CFR 1.10 ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO THE COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450.

(Signature)

James Egan

(Date)

11/3/2003



23914

PATENT TRADEMARK OFFICE

**ALPHA, ALPHA-DISUBSTITUTED BENZYLGLYCINE DERIVATIVES
AS HIV PROTEASE INHIBITORS**

REFERENCE TO RELATED APPLICATIONS

5

This application claims the benefit of U.S. Provisional Application Serial Number 60/426,223 filed November 14, 2002.

10

FIELD OF THE INVENTION

This invention relates generally to alpha, alpha-disubstituted benzylglycine derivatives useful as HIV protease inhibitors, pharmaceutical compositions and
15 diagnostic kits including the same, and methods for using the same for treating viral infection or an assay standards or reagents.

BACKGROUND OF THE INVENTION

20

Two distinct retroviruses, human immunodeficiency virus (HIV) type-1 (HIV-1) or type-2 (HIV-2), have been etiologically linked to the immunosuppressive disease, acquired immunodeficiency syndrome (AIDS). HIV
25 seropositive individuals are initially asymptomatic but typically develop AIDS related complex (ARC) followed by AIDS. Affected individuals exhibit severe immunosuppression, which predisposes them to debilitating and ultimately fatal opportunistic infections.

30

The disease AIDS is the end result of an HIV-1 or HIV-2 virus following its own complex life cycle. The

virion life cycle begins with the virion attaching itself to the host human T-4 lymphocyte immune cell through the bonding of a glycoprotein on the surface of the virion's protective coat with the CD4 glycoprotein on the lymphocyte cell. Once attached, the virion sheds its glycoprotein coat, penetrates into the membrane of the host cell, and uncoats its RNA. The virion enzyme, reverse transcriptase, directs the process of transcribing the RNA into single-stranded DNA. The viral RNA is degraded and a second DNA strand is created. The now double-stranded DNA is integrated into the human cell's genes and those genes are used for virus reproduction.

At this point, RNA polymerase transcribes the integrated DNA into viral RNA. The viral RNA is translated into the precursor *gag-pol* fusion polyprotein. The polyprotein is then cleaved by the HIV protease enzyme to yield the mature viral proteins. Thus, HIV protease is responsible for regulating a cascade of cleavage events that lead to the virus particle's maturing into a virus that is capable of full infectivity.

The typical human immune system response, killing the invading virion, is taxed because the virus infects and kills the immune system's T cells. In addition, viral reverse transcriptase, the enzyme used in making a new virion particle, is not very specific, and causes transcription mistakes that result in continually changed glycoproteins on the surface of the viral protective coat. This lack of specificity decreases the immune

system's effectiveness because antibodies specifically produced against one glycoprotein may be useless against another, hence reducing the number of antibodies available to fight the virus. The virus continues to reproduce while the immune response system continues to weaken. Eventually, the HIV largely holds free reign over the body's immune system, allowing opportunistic infections to set in and without the administration of antiviral agents, immunomodulators, or both, death may result.

There are at least three critical points in the virus's life cycle which have been identified as possible targets for antiviral drugs: (1) the initial attachment of the virion to the T-4 lymphocyte or macrophage site, (2) the transcription of viral RNA to viral DNA (reverse transcriptase, RT), and (3) the processing of gag-pol protein by HIV protease.

The genomes of retroviruses encode a protease that is responsible for the proteolytic processing of one or more polyprotein precursors such as the *pol* and *gag* gene products. See Willink, *Arch. Virol.* 98 1 (1988). Retroviral proteases most commonly process the *gag* precursor into the core proteins, and also process the *pol* precursor into reverse transcriptase and retroviral protease.

The correct processing of the precursor polyproteins by the retroviral protease is necessary for the assembly of the infectious virions. It has been shown that *in vitro* mutagenesis that produces protease-defective virus

leads to the production of immature core forms which lack infectivity. See Crawford et al., *J. Virol.* 53 899 (1985); Kato et al., *Virology* 145 280 (1985).

Therefore, retroviral protease inhibition provides an
5 attractive target for antiviral therapy. See Mitsuya, *Nature* 325 775 (1987).

As evidenced by the protease inhibitors presently marketed and in clinical trials, a wide variety of
10 compounds have been studied as potential HIV protease inhibitors. Hydroxyethylamino-sulfonamides have been disclosed in the literature. See, for example, Kaltenbach and Trainor U.S. Pat. No. 6,391,919, 2002; Vazquez et al. U.S. Pat. No. 6,156,768, 2000; Getman et al. U.S. Pat.
15 No. 6,150,556, 2000; Getman et al. U.S. Pat. No. 6,143,788, 2000; Vazquez et al. U.S. Pat. No. 6,046,190, 2000; Getman et al. U.S. Pat. No. 5,776,971, 1998; and Getman et al. U.S. Pat. No. 5,756,533, 1998. These disclosures do not teach or suggest the compounds of this
20 invention.

SUMMARY OF THE INVENTION

Accordingly, one object of the present invention is
25 to provide novel protease inhibitors.

It is another object of the present invention to provide a novel method for treating HIV infection which comprises administering to a host in need of such
30 treatment a therapeutically effective amount of at least one of the compounds of the present invention or a pharmaceutically acceptable salt form thereof.

It is another object of the present invention to provide a novel method for treating HIV infection which comprises administering to a host in need thereof a therapeutically effective combination of (a) one of the
5 compounds of the present invention and (b) another therapeutic agent (e.g., one or more compounds selected from the group HIV reverse transcriptase inhibitors and HIV protease inhibitors).

10 It is another object of the present invention to provide pharmaceutical compositions with protease inhibiting activity comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of at least one of the compounds of the present invention
15 or a pharmaceutically acceptable salt form thereof.

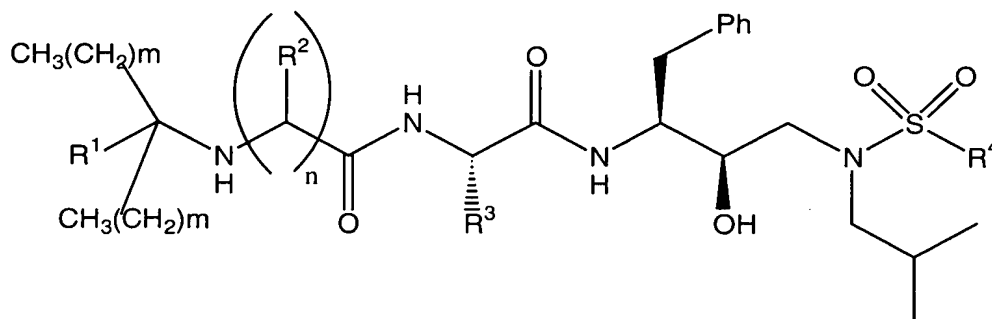
It is another object of the present invention to provide a method of inhibiting HIV present in a body fluid sample, which comprises treating the body fluid
20 sample with an effective amount of a compound of the present invention.

It is another object of the present invention to provide novel kit or container comprising at least one of
25 the compounds of the present invention in an amount effective for use as a standard or reagent in a test or assay for determining the ability of a potential pharmaceutical to inhibit HIV protease, HIV growth, or both.

30

These and other objects, which will become apparent during the following detailed description, have been

achieved by the inventors' discovery that compounds of Formula (I):



5

(I)

wherein:

R^1 is phenyl, pyridinyl, pyrimidinyl, pyrazinyl, or pyridazinyl, and is unsubstituted or substituted with 1-2
 10 substituents selected from the group consisting of halogen, C_{1-6} alkyl, trifluoromethyl, C_{1-6} alkoxy, trifluoromethoxy, and cyano;

R^2 is independently hydrogen or C_{1-6} alkyl;

15

R^3 is selected from isopropyl, tert-butyl, sec-butyl, and $\text{C}(\text{CH}_3)_2\text{SCH}_3$;

R^4 is phenyl, indazolyl, benzothiazolyl, quinolinyl, quinoxalinyl, 2,3-dihydrobenzofuranyl, or
 20 1,3-benzodioxolyl, and is unsubstituted or substituted with 1-2 substituents selected from the group consisting of amino, acetamido, halo, C_{1-6} alkyl, trifluoromethyl, C_{1-6} alkoxy, trifluoromethoxy, and cyano;

25

m is independently selected from 0, 1, 2, or 3; and

n is 1 or 2;

or a pharmaceutically acceptable salt thereof,

- 5 are effective protease inhibitors; inhibit HIV protease, HIV growth, or both.

In another embodiment, the present invention provides compounds of Formula I where R¹ is phenyl,
10 3-fluorophenyl, 4-fluorophenyl, 3,5-difluorophenyl, 2,4-difluorophenyl, 2,6-difluorophenyl, 3-trifluoromethylphenyl, 3-pyridinyl, or 3-methoxyphenyl.

In another embodiment, the present invention
15 provides compounds of Formula I where R¹ is phenyl, 3-fluorophenyl, 4-fluorophenyl, 3,5-difluorophenyl, or 3-methoxyphenyl.

In another embodiment, the invention provides
20 compounds of Formula I where R² is hydrogen or methyl.

In another embodiment, the present invention provides compounds of Formula I where R⁴ is selected from the group consisting of 4-aminophenyl, 3-aminophenyl,
25 3-amino-4-methylphenyl, 4-methoxyphenyl, 6-benzothiazolyl, 2-amino-6-benzothiazolyl, 2-acetamido-6-benzothiazolyl, 2-methyl-6-benzothiazolyl, 7-benzothiazolyl, 2-amino-7-benzothiazolyl, 2-acetamido-7-benzothiazolyl, 2-methyl-7-benzothiazolyl,
30 2,3-dihydrobenzofuran-5-yl, 2,3-benzodioxol-5-yl, 6-indazolyl, 6-quinolinyl, and 6-quinoxaliny.

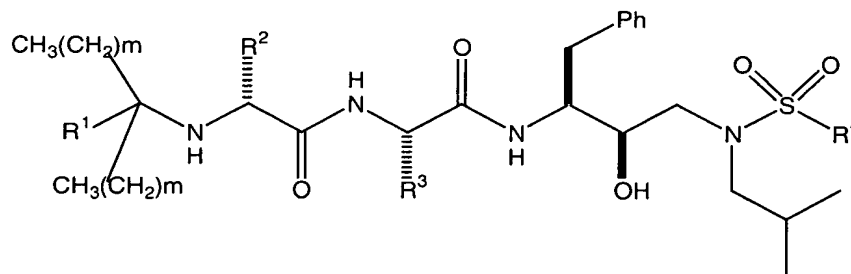
In another embodiment, the present invention provides compounds of Formula I where R^4 is selected from 4-aminophenyl, 6-indazolyl, 6-benzothiazolyl, 2-amino-6-benzothiazolyl, 6-quinolinyl, and 4-methyl-3-aminophenyl.

In another embodiment, the present invention provides compounds of Formula I where m is 0.

10 In another embodiment, the present invention provides compounds of Formula I where n is 1.

In another embodiment, the present invention provides compounds of Formula Ia.

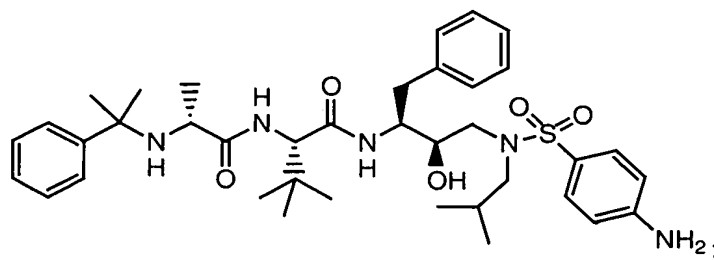
15

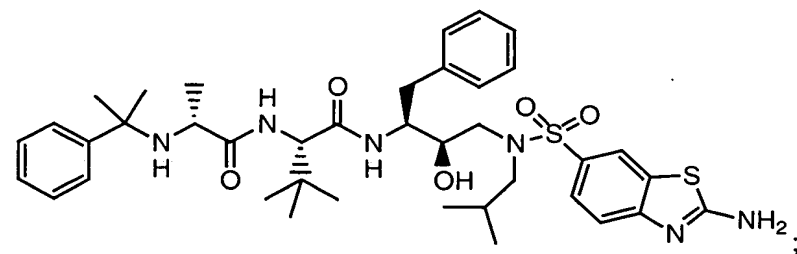
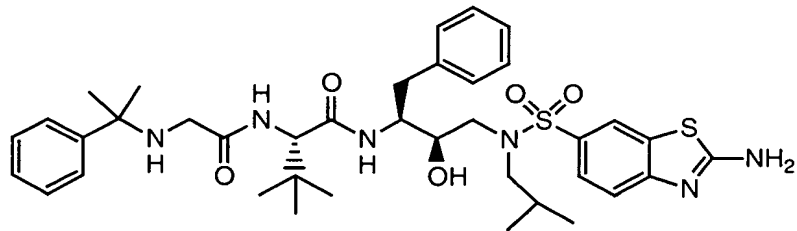
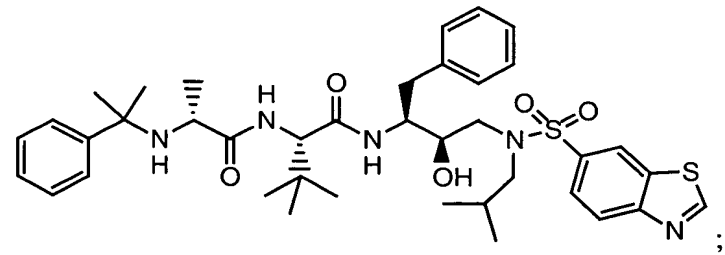
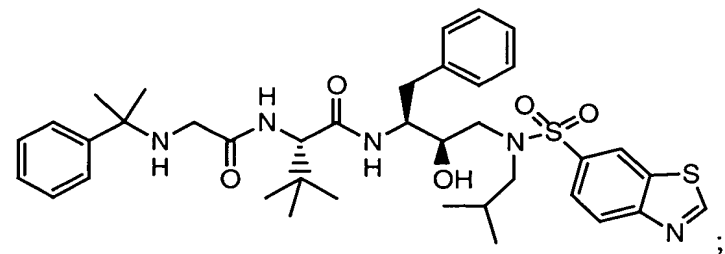
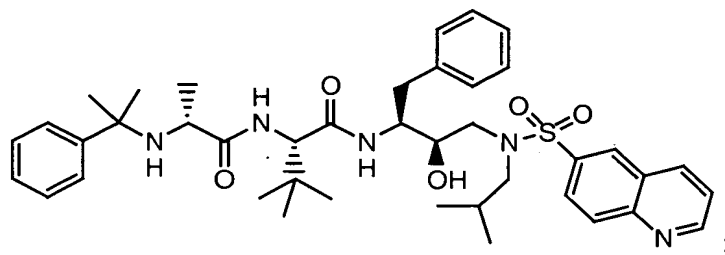
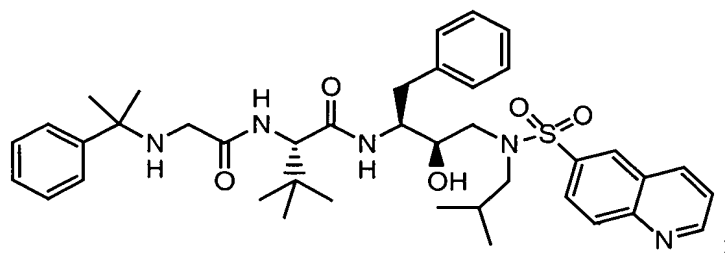


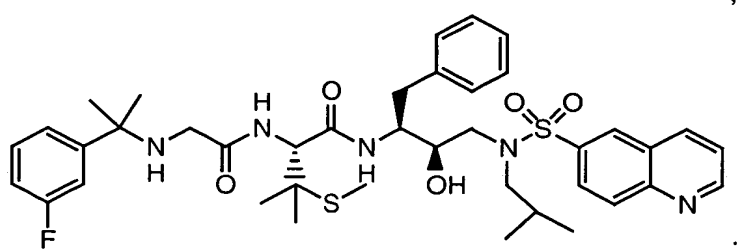
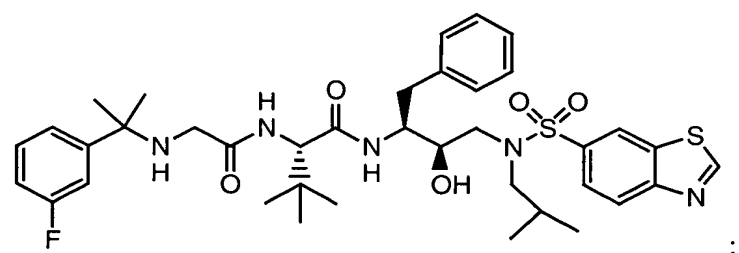
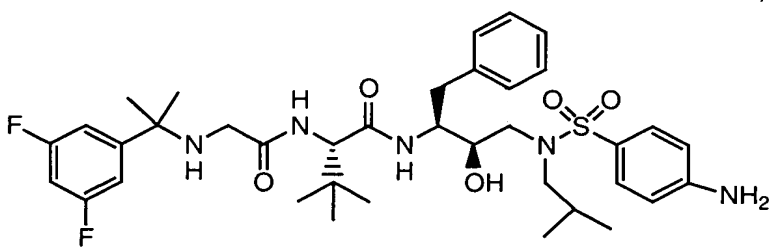
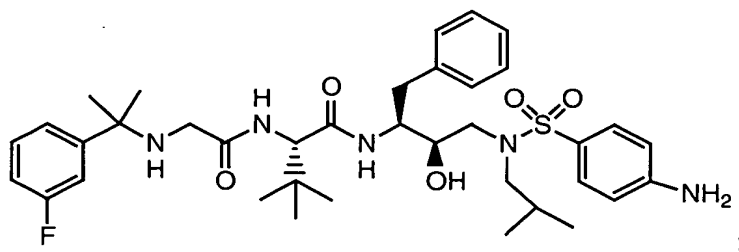
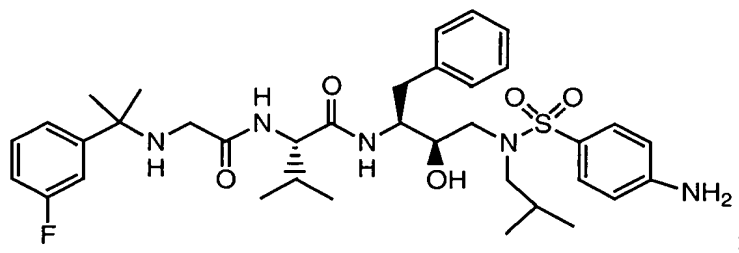
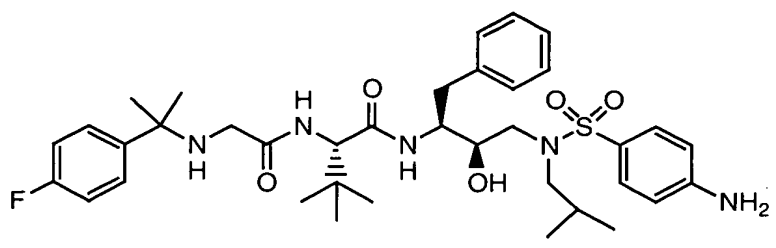
Ia

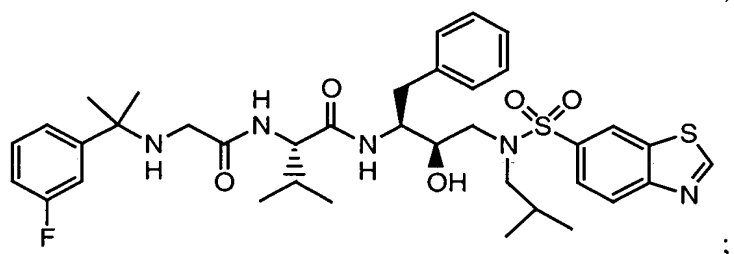
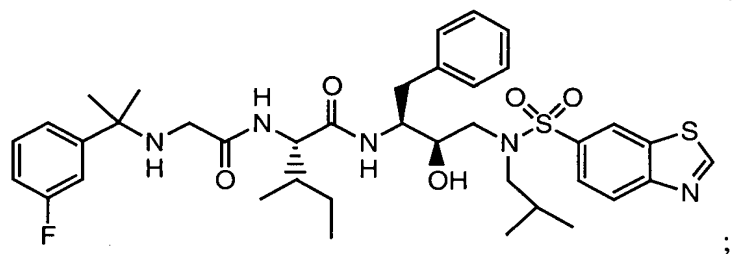
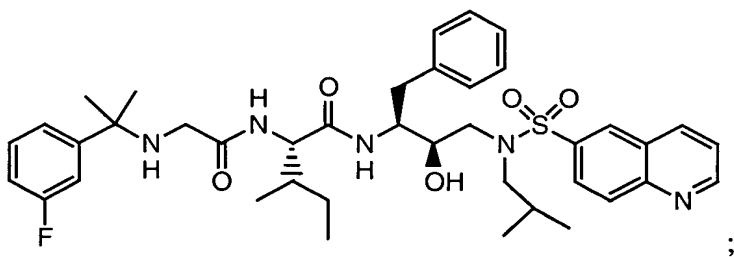
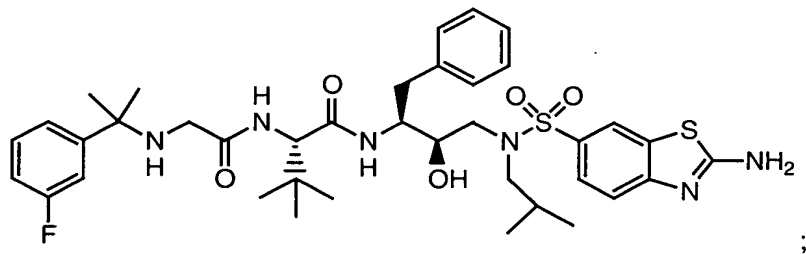
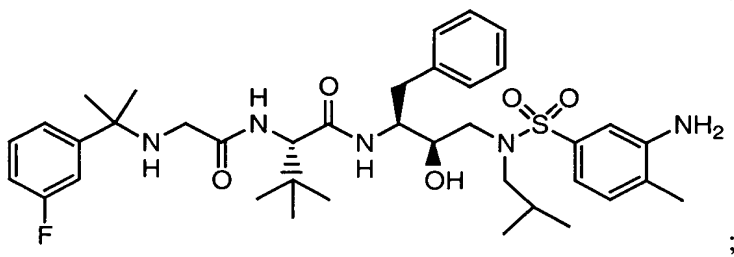
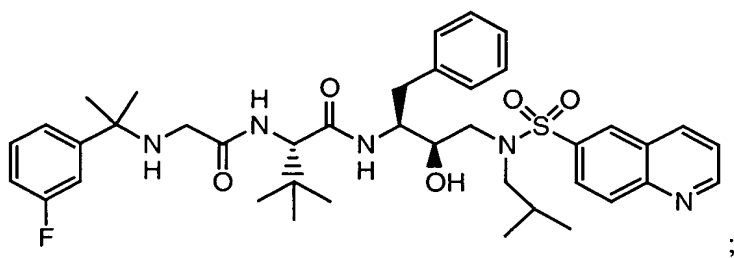
Some compounds of the invention include the following:

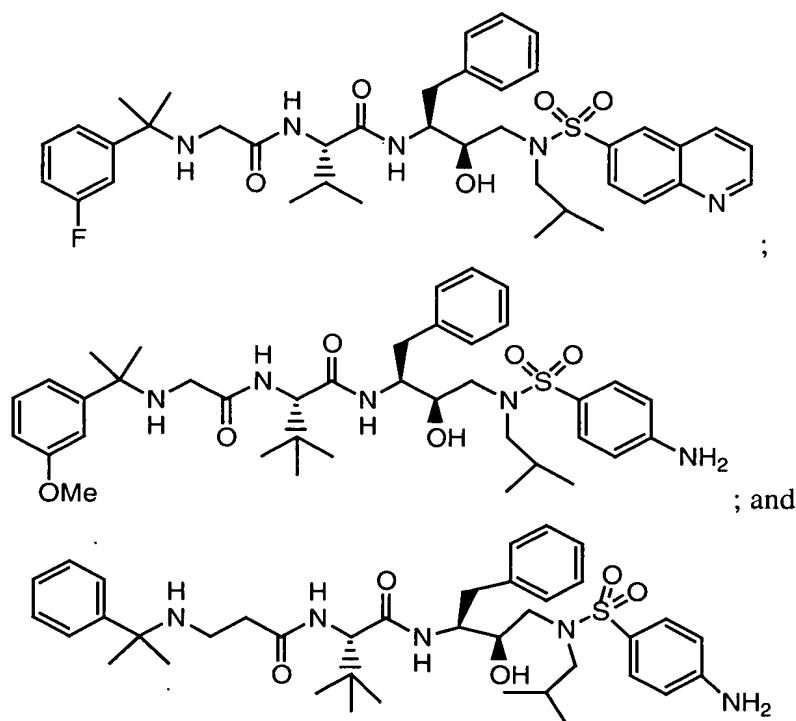
20











5 or pharmaceutically acceptable salts thereof.

In another embodiment, the present invention provides a novel pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound of Formula (I) or pharmaceutically acceptable salt form thereof.

In another embodiment, the present invention provides a novel method for treating HIV infection which comprises administering to a host in need of such treatment a therapeutically effective amount of a compound of Formula (I) or stereoisomeric form, mixtures of stereoisomeric forms, or pharmaceutically acceptable salts thereof.

In another embodiment, the present invention provides a novel method of treating HIV infection which comprises administering, in combination, to a host in need thereof a therapeutically effective amount of:

5

- (a) a compound of Formula (I); and,
- (b) at least one compound selected from the group HIV reverse transcriptase inhibitors and HIV protease inhibitors.

10

In another embodiment, the reverse transcriptase inhibitor is selected from the group AZT, ddC, ddI, d4T, 3TC, delavirdine, efavirenz, nevirapine, Ro 18, 893, trovirdine, MKC-442, HBY 097, ACT, UC-781, UC-782, RD4-
15 2025, and MEN 10979, and the protease inhibitor is selected from the group saquinavir, ritonavir, indinavir, amprenavir, nelfinavir, palinavir, BMS-232623, GS3333, KNI-413, KNI-272, LG-71350, CGP-61755, PD 173606, PD 177298, PD 178390, PD 178392, U-140690, and ABT-378.

20

In another embodiment, the reverse transcriptase inhibitor is selected from the group AZT, efavirenz, and 3TC and the protease inhibitor is selected from the group saquinavir, ritonavir, nelfinavir, and indinavir.

25

In another embodiment, the reverse transcriptase inhibitor is AZT.

In another embodiment, the protease inhibitor is
30 indinavir.

In another, component (b) is a HIV reverse transcriptase inhibitor and a HIV protease inhibitor.

In another embodiment, component (b) is two
5 different HIV reverse transcriptase inhibitors.

In another embodiment, the present invention provides a pharmaceutical composition useful for the treatment of HIV infection, which comprises a
10 therapeutically effective amount of:

- (a) a compound of Formula (I); and,
- (b) at least one compound selected from the group
HIV reverse transcriptase inhibitors and HIV
15 protease inhibitors.

In another embodiment, the present invention provides a novel method of inhibiting HIV present in a body fluid sample which comprises treating the body fluid
20 sample with an effective amount of a compound of Formula (I).

In another embodiment, the present invention to provides a novel a kit or container comprising a compound
25 of Formula (I) in an amount effective for use as a standard or reagent in a test or assay for determining the ability of a potential pharmaceutical to inhibit HIV protease, HIV growth, or both.

30 In another embodiment, the present invention provides a compound of Formula (I) for use in medical therapy.

In another embodiment, the present invention provides the use of a compound of Formula (I) for the manufacture of a medicament for treating HIV infection.

5

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following terms and expressions have the indicated meanings. It will be appreciated that the compounds of the present invention contain

10 asymmetrically substituted carbon atoms, and may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms or by synthesis, from optically active starting materials. All

15 chiral, diastereomeric, racemic forms and all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomeric form is specifically indicated. With the center bearing R^2 , this center can be non-chiral (i.e., each R^2 can be the same), or this

20 center can be either (R)- or (S)-. Additionally, with the center bearing two $CH_3(CH_2)_m$ groups, this center can be non-chiral (i.e., each m can be the same), or this center can be either (R)- or (S)-.

25 As used herein, "HIV reverse transcriptase inhibitor" is intended to refer to both nucleoside and non-nucleoside inhibitors of HIV reverse transcriptase (RT). Examples of nucleoside RT inhibitors include, but are not limited to, AZT, ddC, ddI, d4T, and 3TC. Example

30 of non-nucleoside RT inhibitors include, but are not limited to, delavirdine (Pharmacia and Upjohn, U90152S), efavirenz (Bristol-Myers Squibb), nevirapine (Boehringer

Ingelheim), Ro 18,893 (Roche), trovirdine (Lilly), MKC-442 (Triangle), HBY 097 (Hoeschst), HBY 1293 (Hoeschst), ACT (Korean Research Institute), UC-781 (Rega Institute), UC-782 (Rega Institute), RD4-2025 (Tosoh Co. Ltd.), and
5 MEN 10979 (Menarini Farmaceutici).

As used herein, "HIV protease inhibitor" is intended to refer to compounds which inhibit HIV protease. Examples include, but are not limited, saquinavir (Roche, Ro31-8959), ritonavir (Abbott, ABT-538), indinavir (Merck, MK-639), amprenavir (Vertex/Glaxo Wellcome), nelfinavir (Agouron, AG-1343), palinavir (Boehringer Ingelheim), BMS-232623 (Bristol-Myers Squibb), GS3333 (Gilead Sciences), KNI-413 (Japan Energy), KNI-272 (Japan
15 Energy), LG-71350 (LG Chemical), CGP-61755 (Ciba-Geigy), PD 173606 (Parke Davis), PD 177298 (Parke Davis), PD 178390 (Parke Davis), PD 178392 (Parke Davis), tipranavir (Pharmacia and Upjohn, U-140690), DMP-450 (Bristol-Myers Squibb) and ABT-378.

20

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable
25 salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the
30 quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include

those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, 5 lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

10

The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound, which contains a basic or acidic moiety, by conventional chemical methods. Generally, such salts can be prepared 15 by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or 20 acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

25 The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals 30 without excessive toxicity, irritation, allergic response, or other problem or complication commensurate with a reasonable benefit/risk ratio.

"Stable compound" and "stable structure" are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent. Only stable compounds are contemplated by the present invention.

"Substituted" is intended to indicate that one or more (e.g., 1, 2, 3, 4, or 5; preferably 1, 2, or 3; and more preferably 1 or 2) hydrogens on the group indicated in the expression using "substituted" is replaced with a selection from the indicated group(s), provided that the indicated atom's normal valency is not exceeded, and that the substitution results in a stable compound. For example, "substituted aryl" is intended to mean an "aryl" group substituted by one or more (e.g., 1, 2, 3, 4, or 5; preferably 1, 2, or 3; and more preferably 1 or 2) indicated group(s). When a substituent is keto (i.e., =O) or thioxo (i.e., =S) group, then 2 hydrogens on an atom of the indicated group are replaced.

"Therapeutically effective amount" is intended to include an amount of a compound of the present invention or an amount of the combination of compounds claimed effective to inhibit HIV infection or treat the symptoms of HIV infection in a host. The combination of compounds is preferably a synergistic combination. Synergy, as described for example by Chou and Talalay, Adv. Enzyme Regul. 22:27-55 (1984), occurs when the effect (in this case, inhibition of HIV replication) of the compounds when administered in combination is greater than the additive effect of the compounds when administered alone

as a single agent. In general, a synergistic effect is most clearly demonstrated at suboptimal concentrations of the compounds. Synergy can be in terms of lower cytotoxicity, increased antiviral effect, or some other
5 beneficial effect of the combination compared with the individual components.

One diastereomer of a compound of Formula (I) may display superior activity compared with the other. When
10 required, separation of the racemic material can be achieved by HPLC using a chiral column or by a resolution using a resolving agent such as camphonic chloride as in Thomas J. Tucker, et al., *J. Med. Chem.* **1994** 37, 2437-2444. A chiral compound of Formula (I) may also be
15 directly synthesized using a chiral catalyst or a chiral ligand, e.g. Mark A. Huffman, et al., *J. Org. Chem.* **1995**, 60, 1590-1594.

The term "alkyl" refers to a monoradical branched or
20 unbranched saturated hydrocarbon chain preferably having 1, 2, 3, 4, 5, or 6 carbon atoms, and more preferably from 1, 2, 3, or 4 carbon atoms. Examples of alkyl include, but are not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *sec*-butyl, *n*-hexyl, and
25 the like. Preferred "alkyl" group, unless otherwise specified, is methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, and *sec*-butyl.

The term "substituted alkyl" refers to an "alkyl"
30 group which is substituted, for example, with one or more (preferably 1, 2, 3, 4, or 5; more preferably 1, 2, or 3) substituents, independently selected from methyl, ethyl,

alkenyl, alkynyl, alkoxy, trifluoromethoxy, halo,
haloalkyl, hydroxy, hydroxyalkyl, carboxyalkyl,
difluoromethyl, trifluoromethyl, nitro, cyano, carboxy,
alkanoyl, alkoxycarbonyl, amido, alkylamido,
5 dialkylamido, amino, alkylamino, dialkylamino, acylamino,
sulfonylamino, alkylthio, trifluoromethylthio,
alkylsulfinyl, alkylsulfonyl, sulfonamido,
alkylsulfonamido, dialkylsulfonamido, aryl, substituted
aryl, heteroaryl, substituted heteroaryl, heterocycle,
10 substituted heterocycle, cycloalkyl, substituted
cycloalkyl, keto, and thioxo.

The term "alkenyl", as used herein, is intended to
include monoradical hydrocarbon chains of either a
15 straight or branched configuration and one or more
unsaturated carbon-carbon bonds which may occur in any
stable point along the chain preferably having 2, 3, 4,
5, or 6 carbon atoms, more preferably from 2, 3, or 4
carbon atoms. This term is exemplified by groups such as
20 ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-
butenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, and the like.

The term "alkynyl" refers to a monoradical
hydrocarbon chain of either a straight or branched
25 configuration and one or more carbon-carbon triple bonds
which may occur in any stable point along the chain,,
preferably having from 2, 3, 4, 5, or 6 carbon atoms,
and more preferably from 2, 3 or 4 carbon atoms. This
term is exemplified by groups such as ethynyl, 1-
30 propynyl, 2-propynyl, 1-butyne, 2-butyne, 3-butyne, 1-
hexynyl, 2-hexynyl, 3-hexynyl, and the like.

The term "alkoxy" refers to the groups alkyl-O-, where alkyl is defined herein. Preferred alkoxy groups include, e.g., methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 5 1,2-dimethylbutoxy, and the like. Preferred alkoxy groups are methoxy, ethoxy, *n*-propoxy, *i*-propoxy, *n*-butoxy, *s*-butoxy, and *t*-butoxy.

The term "aryl" refers to an unsaturated aromatic 10 carbocyclic group of from 6 to 12 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings, wherein at least one ring is aromatic (e.g., naphthyl, tetrahydromaphthyl, dihydrophenanthrenyl, fluorenyl, or anthryl). Preferred aryls include phenyl, 15 naphthyl and the like.

The term "substituted aryl" refers to an "aryl" group which is substituted, for example, with one or more, and in particular one to three, substituents, 20 independently selected from methyl, ethyl, alkenyl, alkynyl, alkoxy, trifluoromethoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, carboxyalkyl, difluoromethyl, trifluoromethyl, nitro, cyano, carboxy, alkanoyl, alkoxycarbonyl, amido, alkylamido, dialkylamido, amino, 25 alkylamino, dialkylamino, acylamino, sulfonylamino, alkylthio, trifluoromethylthio, alkylsulfinyl, alkylsulfonyl, sulfonamido, alkylsulfonamido, and dialkylsulfonamido.

30 The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 10 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups

include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like. Preferred
5 cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

The term "substituted cycloalkyl" refers to a "cycloalkyl" group which is substituted, for example,
10 with one or more, and in particular 1, 2, or 3, substituents, independently selected from alkyl, alkenyl, alkynyl, alkoxy, trifluoromethoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, carboxyalkyl, difluoromethyl, trifluoromethyl, nitro, cyano, carboxy, alkanoyl,
15 alkoxycarbonyl, amido, alkylamido, dialkylamido, amino, alkylamino, dialkylamino, acylamino, sulfonylamino, alkylthio, trifluoromethylthio, alkylsulfinyl, alkylsulfonyl, sulfonamido, alkylsulfonamido, dialkylsulfonamido, keto, and thioxo.

20

The term "halo" or "halogen" refers to fluoro, chloro, bromo, or iodo. Unless otherwise specified, preferred halo is fluoro or chloro.

25 "Haloalkyl" refers to alkyl as defined herein substituted by 1, 2, 3, 4, or 5 halo groups as defined herein, which may be the same or different. Representative haloalkyl groups include, by way of example, difluoromethyl, trifluoromethyl,
30 trichloromethyl, pentafluoroethyl, pentachloroethyl, 2,2,2-trifluoroethyl, 2,2-difluoroethyl, and the like.

Preferred haloalkyl is selected from difluoromethyl and trifluoromethyl.

The term "heteroaryl" is defined herein as a
5 monocyclic, bicyclic, or tricyclic ring system containing one, two, or three aromatic rings and containing at least one nitrogen, oxygen, or sulfur atom in an aromatic ring.

Examples of heteroaryl groups include, but are not
10 limited to, 2*H*-pyrrolyl, 3*H*-indolyl, 4*H*-quinolizinyll, 4*nH*-carbazolyl, acridinyl, benzo[*b*]thienyl, benzothiazolyl, β -carbolinyl, carbazolyl, chromenyl, cinnolinyl, dibenzo[*b,d*]furanyl, furazanyl, furyl, imidazolyl, imidizolyl, indazolyl, indolisinyl, indolyl,
15 isobenzofuranyl, isoindolyl, isoquinolyl, isothiazolyl, isoxazolyl, naphthyridinyl, naphtho[2,3-*b*], oxazolyl, perimidinyl, phenanthridinyl, phenanthrolinyl, phenarsazinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, pteridinyl,
20 purinyl, pyranyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridyl, pyrimidinyl, pyrimidinyl, pyrrolyl, quinazolinyl, quinolyl, quinoxalinyl, thiadiazolyl, thianthrenyl, thiazolyl, thienyl, triazolyl, and xanthenyl. In one embodiment the term "heteroaryl"
25 denotes a monocyclic aromatic ring containing five or six ring atoms containing carbon and 1, 2, 3, or 4 heteroatoms independently selected from the group non-peroxide oxygen, sulfur, and N(Z) wherein Z is absent or is H, O, alkyl, phenyl or benzyl. In another embodiment
30 heteroaryl denotes an ortho-fused bicyclic heterocycle of about eight to ten ring atoms derived therefrom,

particularly a benz-derivative or one derived by fusing a propylene, or tetramethylene diradical thereto.

The term "substituted heteroaryl" is defined herein
5 as a "heteroaryl" group which is substituted, for
example, with one or more, and in particular 1, 2, or 3,
substituents, independently selected from alkyl, alkenyl,
alkynyl, alkoxy, trifluoromethoxy, halo, haloalkyl,
hydroxy, hydroxyalkyl, carboxyalkyl, difluoromethyl,
10 trifluoromethyl, nitro, cyano, carboxy, alkanoyl,
alkoxycarbonyl, amido, alkylamido, dialkylamido, amino,
alkylamino, dialkylamino, acylamino, sulfonylamino,
alkylthio, trifluoromethylthio, alkylsulfinyl,
alkylsulfonyl, sulfonamido, alkylsulfonamido, and
15 dialkylsulfonamido.

The term "heterocycle" refers to a saturated or
partially unsaturated ring system, containing at least
one heteroatom selected from the group oxygen, nitrogen,
20 and sulfur. Typically heterocycle is a monocyclic,
bicyclic, or tricyclic group containing one or more
heteroatoms selected from the group oxygen, nitrogen, and
sulfur. A heterocycle group also can contain an oxo
group (=O) attached to the ring. Non-limiting examples
25 of heterocycle groups include 1,3-dihydrobenzofuran, 1,3-
dioxolane, 1,4-dioxane, 1,4-dithiane, 2*H*-pyran, 2-
pyrazoline, 4*H*-pyran, chromanyl, imidazolidinyl,
imidazolinyl, indolinyl, isochromanyl, isoindolinyl,
morpholine, piperazinyl, piperidine, piperidyl,
30 pyrazolidine, pyrazolidinyl, pyrazolinyl, pyrrolidine,
pyrroline, quinuclidine, and thiomorpholine.

The term "substituted heterocycle" refers to a "heterocycle" group, as defined herein, which is substituted with one or more, and in particular one to three, substituents, selected from alkyl, alkenyl, alkynyl, alkoxy, halo, haloalkyl, hydroxy, hydroxyalkyl, aryl, heteroaryl, cycloalkyl, alkanoyl, alkoxycarbonyl, amino, alkylamino, dialkylamino, trifluoromethylthio, difluoromethyl, acylamino, nitro, trifluoromethyl, trifluoromethoxy, carboxy, carboxyalkyl, keto, thioxo, alkylthio, alkylsulfinyl, alkylsulfonyl and cyano.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

Preferred 5 to 6 membered heterocycles include, but are not limited to, pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, furanyl, thienyl, thiazolyl, pyrrolyl, piperazinyl, piperidinyl, pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, tetrazolyl; more preferred 5 to 6 membered heterocycles include, but are not limited to, pyridinyl, pyrimidinyl, triazinyl,

furanyl, thienyl, thiazolyl, piperaziny, piperidinyl, pyrazolyl, imidazolyl, and tetrazolyl.

The term "alkanoyl" refers to $-C(=O)R^a$, wherein R^a is an alkyl group as previously defined.

The term "alkoxycarbonyl" refers to $-C(=O)OR^a$, wherein R^a is an alkyl group as previously defined.

The term "amino" refers to $-NH_2$.

The term "alkylamino" refers to $-NHR^a$, wherein R^a is an alkyl group as previously defined.

The term "dialkylamino" refers to $-N(R^a)_2$ wherein each R^a is an alkyl group as previously defined and may be the same or different.

The term "amido" refers to $-C(=O)NH_2$.

The term "alkylamido" refers to $-C(=O)NHR^a$, wherein R^a is an alkyl group as previously defined.

The term "dialkylamido" refers to $-C(=O)N(R^a)_2$ wherein each R^a is an alkyl group as previously defined and may be the same or different.

The term "acylamino" refers to $-NHC(=O)R^b$, wherein R^b is an alkyl group or an aryl group as previously defined.

The term "sulfonylamino" refers to $\text{-NHS(=O)}_2\text{R}^b$, wherein R^b is an alkyl group or an aryl group as previously defined.

5 The term "alkylthio" refers to the groups alkyl-S-, wherein alkyl is as previously defined herein.

The term "alkylsulfinyl" refers to -S(=O)R^a , wherein R^a is an alkyl group as previously defined.

10

The term "alkylsulfonyl" refers to $\text{-S(=O)}_2\text{R}^a$, wherein R^a is an alkyl group as previously defined.

The term "sulfonamido" refers to $\text{-S(=O)}_2\text{NH}_2$.

15

The term "alkylsulfonamido" refers to $\text{-S(=O)}_2\text{NHR}^a$, wherein R^a is an alkyl group as previously defined.

20 The term "dialkylsulfonamido" refers to $\text{-S(=O)}_2\text{N(R}^a)_2$ wherein each R^a is an alkyl group as previously defined and may be the same or different.

The term "nitro" refers to -NO_2 .

25 The term "difluoromethyl" refers to -CHF_2 .

The term "trifluoromethyl" refers to -CF_3 .

The term "trifluoromethoxy" refers to -OCF_3 .

30

The term "trifluoromethylthio" refers to -SCF_3 .

The term "cyano" refers to -CN.

The term "hydroxy" refers to -OH.

5 The term "hydroxyalkyl" refers to an alkyl group as defined above substituted by a hydroxy group.

The term "carboxyalkyl" refers to an alkyl group as defined above substituted by a -CO₂H group.

10

The term "carboxy" refers to -CO₂H.

As to any of the above groups, which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

20

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

25

As used herein, "μg" denotes microgram, "mg" denotes milligram, "g" denotes gram, "μL" denotes microliter, "mL" denotes milliliter, "L" denotes liter, "nM" denotes nanomolar, "μM" denotes micromolar, "mM" denotes millimolar, "M" denotes molar and "nm" denotes nanometer. "Sigma" stands for the Sigma-Aldrich Corp. of St. Louis MO.

30

Utility

The compounds of Formula (I) possess HIV protease inhibitory activity and are therefore useful as antiviral agents for the treatment of HIV infection and associated diseases. The compounds of Formula (I) possess HIV protease inhibitory activity and are effective as inhibitors of HIV growth. The ability of the compounds of the present invention to inhibit viral growth or infectivity is demonstrated in standard assay of viral growth or infectivity, for example, using the assay described below.

The compounds of Formula (I) of the present invention are also useful for the inhibition of HIV in an *ex vivo* sample containing HIV or expected to be exposed to HIV. Thus, the compounds of the present invention may be used to inhibit HIV present in a body fluid sample (for example, a serum or semen sample), which contains or is suspected to contain or be exposed to HIV.

The compounds provided by this invention are also useful as standard or reference compounds for use in tests or assays for determining the ability of an agent to inhibit viral clone replication and/or HIV protease, for example in a pharmaceutical research program. Thus, the compounds of the present invention may be used as control or reference compound in such assays and as a quality control standard. The compounds of the present invention may be provided in a commercial kit or container for use as such standard or reference compound.

Since the compounds of the present invention exhibit specificity for HIV protease, the compounds of the present invention may also be useful as diagnostic reagents in diagnostic assays for the detection of HIV protease. Thus, inhibition of the protease activity in an assay (such as the assays described herein) by a compound of the present invention would be indicative of the presence of HIV protease and HIV virus.

10

HIV RNA Assay

DNA Plasmids and *in vitro* RNA transcripts:

Plasmid pDAB 72 containing both gag and pol sequences of BH10 (bp 113-1816) cloned into PTZ 19R was prepared according to Erickson-Vitanen et al. *AIDS Research and Human Retroviruses* **1989**, 5, 577. The plasmid was linearized with Bam HI prior to the generation of *in vitro* RNA transcripts using the Riboprobe Gemini system II kit (Promega) with T7 RNA polymerase. Synthesized RNA was purified by treatment with RNase free DNase (Promega), phenol-chloroform extraction, and ethanol precipitation. RNA transcripts were dissolved in water, and stored at -70EC. The concentration of RNA was determined from the A₂₆₀.

Probes:

Biotinylated capture probes were purified by HPLC after synthesis on an Applied Biosystems (Foster city, CA) DNA synthesizer by addition of biotin to the 5N terminal end of the oligonucleotide, using the biotin-

phosphoramidite reagent of Cocuzza, *Tet. Lett.* **1989**, 30, 6287. The gag biotinylated capture probe (5-biotin-CTAGCTCCCTGCTTGCCCATACTA 3N) was complementary to nucleotides 889-912 of HXB2 and the pol biotinylated
5 capture probe (5N-biotin -CCCTATCATTTTTGGTTTCCAT 3N) was complementary to nucleotides 2374-2395 of HXB2. Alkaline phosphatase conjugated oligonucleotides used as reporter probes were prepared by Syngene (San Diego, CA). The pol
10 reporter probe (5N CTGTCTTACTTTGATAAAACCTC 3N) was complementary to nucleotides 2403-2425 of HXB2. The gag reporter probe (5N CCCAGTATTTGTCTACAGCCTTCT 3N) was complementary to nucleotides 950-973 of HXB2. All nucleotide positions are those of the GenBank Genetic Sequence Data Bank as accessed through the Genetics
15 Computer Group Sequence Analysis Software Package (Devereau *Nucleic Acids Research* **1984**, 12, 387). The reporter probes were prepared as 0.5 :M stocks in 2 x SSC (0.3 M NaCl, 0.03 M sodium citrate), 0.05 M Tris pH 8.8, 1 mg/mL BSA. The biotinylated capture probes were
20 prepared as 100 :M stocks in water.

Streptavidin coated plates:

Streptavidin coated plates were obtained from Du
25 Pont Biotechnology Systems (Boston, MA).

Cells and virus stocks:

MT-2 and MT-4 cells were maintained in RPMI 1640
30 supplemented with 5% fetal calf serum (FCS) for MT-2 cells or 10% FCS for MT-4 cells, 2 mM L-glutamine and 50 µg/mL gentamycin, all from Gibco. HIV-1 RF was

propagated in MT-4 cells in the same medium. Virus stocks were prepared approximately 10 days after acute infection of MT-4 cells and stored as aliquots at -70°C . Infectious titers of HIV-1(RF) stocks were $1-3 \times 10^7$ PFU (plaque forming units)/mL as measured by plaque assay on MT-2 cells (see below). Each aliquot of virus stock used for infection was thawed only once.

For evaluation of antiviral efficacy, cells to be infected were subcultured one day prior to infection. On the day of infection, cells were resuspended at 5×10^5 cells/mL in RPMI 1640, 5% FCS for bulk infections or at 2×10^6 /mL in Dulbecco's modified Eagles medium with 5% FCS for infection in microtiter plates. Virus was added and culture continued for 3 days at 37°C .

HIV RNA assay:

Cell lysates or purified RNA in 3 M or 5 M GED were mixed with 5 M GED and capture probe to a final guanidinium isothiocyanate concentration of 3 M and a final biotin oligonucleotide concentration of 30 nM. Hybridization was carried out in sealed U bottom 96 well tissue culture plates (Nunc or Costar) for 16-20 hours at 37°C . RNA hybridization reactions were diluted three-fold with deionized water to a final guanidinium isothiocyanate concentration of 1 M and aliquots (150 μL) were transferred to streptavidin coated microtiter plates wells. Binding of capture probe and capture probe-RNA hybrid to the immobilized streptavidin was allowed to proceed for 2 hours at room temperature, after which the plates were washed 6 times with DuPont ELISA plate wash

buffer (phosphate buffered saline (PBS), 0.05% Tween 20.)
A second hybridization of reporter probe to the
immobilized complex of capture probe and hybridized
target RNA was carried out in the washed streptavidin
5 coated well by addition of 120 μ l of a hybridization
cocktail containing 4 X SSC, 0.66% Triton X 100, 6.66%
deionized formamide, 1 mg/mL BSA and 5 nM reporter probe.
After hybridization for one hour at 37°C, the plate was
again washed 6 times. Immobilized alkaline phosphatase
10 activity was detected by addition of 100 μ L of 0.2 mM 4-
methylumbelliferyl phosphate (MUBP, JBL Scientific) in
buffer δ (2.5 M diethanolamine pH 8.9 (JBL Scientific),
10 mM $MgCl_2$, 5 mM zinc acetate dihydrate and 5 mM N-
hydroxyethyl-ethylene-diamine-triacetic acid). The
15 plates were incubated at 37°C. Fluorescence at 450 nm
was measured using a microplate fluorometer (Dynateck)
exciting at 365 nm.

Microplate based compound evaluation in HIV-1 infected

20 MT-2 cells:

Compounds to be evaluated were dissolved in DMSO and
diluted in culture medium to twice the highest
concentration to be tested and a maximum DMSO
25 concentration of 2%. Further three-fold serial dilutions
of the compound in culture medium were performed directly
in U bottom microtiter plates (Nunc). After compound
dilution, MT-2 cells (50 μ L) were added to a final
concentration of 5×10^5 per mL (1×10^5 per well).
30 Cells were incubated with compounds for 30 minutes at
37°C in a CO₂ incubator. For evaluation of antiviral
potency, an appropriate dilution of HIV-1 (RF) virus

stock (50 μ L) was added to culture wells containing cells and dilutions of the test compounds. The final volume in each well was 200 μ L. Eight wells per plate were left uninfected with 50 μ L of medium added in place of virus, while eight wells were infected in the absence of any antiviral compound. For evaluation of compound toxicity, parallel plates were cultured without virus infection.

After 3 days of culture at 37°C in a humidified chamber inside a CO₂ incubator, all but 25 μ L of medium/well was removed from the HIV infected plates. Thirty seven μ L of 5 M GED containing biotinylated capture probe was added to the settled cells and remaining medium in each well to a final concentration of 3 M GED and 30 nM capture probe. Hybridization of the capture probe to HIV RNA in the cell lysate was carried out in the same microplate well used for virus culture by sealing the plate with a plate sealer (Costar), and incubating for 16-20 hrs in a 37°C incubator. Distilled water was then added to each well to dilute the hybridization reaction three-fold and 150 μ L of this diluted mixture was transferred to a streptavidin coated microtiter plate. HIV RNA was quantitated as described above. A standard curve, prepared by adding known amounts of pDAB 72 *in vitro* RNA transcript to wells containing lysed uninfected cells, was run on each microtiter plate in order to determine the amount of viral RNA made during the infection.

In order to standardize the virus inoculum used in the evaluation of compounds for antiviral activity, dilutions of virus were selected which resulted in an

IC₉₀ value (concentration of compound required to reduce the HIV RNA level by 90%) for dideoxycytidine (ddC) of 0.2 µg/mL. IC₉₀ values of other antiviral compounds, both more and less potent than ddC, were reproducible using several stocks of HIV-1 (RF) when this procedure was followed. This concentration of virus corresponded to ~3 x 10⁵ PFU (measured by plaque assay on MT-2 cells) per assay well and typically produced approximately 75% of the maximum viral RNA level achievable at any virus inoculum. For the HIV RNA assay, IC₉₀ values were determined from the percent reduction of net signal (signal from infected cell samples minus signal from uninfected cell samples) in the RNA assay relative to the net signal from infected, untreated cells on the same culture plate (average of eight wells). Valid performance of individual infection and RNA assay tests was judged according to three criteria. It was required that the virus infection should result in an RNA assay signal equal to or greater than the signal generated from 2 ng of pDAB 72 *in vitro* RNA transcript. The IC₉₀ for ddC, determined in each assay run, should be between 0.1 and 0.3 µg/mL. Finally, the plateau level of viral RNA produced by an effective protease inhibitor should be less than 10% of the level achieved in an uninhibited infection. A compound was considered active if its IC₉₀ was found to be less than 1 µM.

Compounds of the invention have demonstrated IC₉₀ of less than 1 µM in the above assay. For antiviral potency tests, all manipulations in microtiter plates, following the initial addition of 2X concentrated compound solution

to a single row of wells, were performed using a Perkin Elmer/Cetus ProPette.

In addition to the above, it is desirable to find
5 new compounds with improved pharmacological
characteristics compared with known HIV protease
inhibitors. For example, it is preferred to find new
compounds with improved HIV protease inhibitory activity
and selectivity for HIV protease versus other enzymes.
10 It is also desirable and preferable to find compounds
with advantageous and improved characteristics in one or
more of the following categories:

- 15 (a) pharmaceutical properties (i.e. solubility,
permeability, amenability to sustained release
formulations);
- (b) dosage requirements (e.g., lower dosages and/or
once-daily dosing);
- 20 (c) factors which decrease blood concentration
peak-to-trough characteristics (i.e. clearance
and/or volume of distribution);
- (d) factors that increase the concentration of
active drug at the receptor (i.e. protein
binding, volume of distribution);
- 25 (e) factors that decrease the liability for
clinical drug-drug interactions (cytochrome
P450 enzyme inhibition or induction);
- (f) factors that decrease the potential for adverse
side-effects (i.e. pharmacological selectivity
30 beyond HIV proteases, potential chemical or
metabolic reactivity, limited CNS penetration);
and

- (g) factors that improve manufacturing costs or feasibility (i.e. difficulty of synthesis, number of chiral centers, chemical stability, ease of handling).

5

Dosage and Formulation

The antiviral compounds of this invention can be administered as treatment for viral infections by any means that produces contact of the active agent with the agent's site of action, i.e., the viral protease, in the body of a mammal. They can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or in a combination of therapeutic agents. They can be administered alone, but preferably are administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

20

The dosage administered will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the age, health and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; and the effect desired. A daily dosage of active ingredient can be expected to be about 0.001 to about 1000 milligrams per kilogram of body weight, with the preferred dose being about 0.1 to about 30 mg/kg.

30

Dosage forms of compositions suitable for administration contain from about 1 mg to about 1000 mg of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be
5 present in an amount of about 0.5-95% by weight based on the total weight of the composition. The active ingredient can be administered orally in solid dosage forms, such as capsules, tablets and powders, or in liquid dosage forms, such as elixirs, syrups and
10 suspensions. It can also be administered parenterally, in sterile liquid dosage forms.

Gelatin capsules contain the active ingredient and powdered carriers, such as lactose, starch, cellulose
15 derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed
20 tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring
25 and flavoring to increase patient acceptance.

In general, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols
30 are suitable carriers for parenteral solutions. Solutions for parenteral administration preferably contain a water soluble salt of the active ingredient,

suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used
5 are citric acid and its salts, and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben and chlorobutanol. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences, supra*,
10 a standard reference text in this field.

Useful pharmaceutical dosage-forms for administration of the compounds of this invention can be illustrated as follows:

15

Capsules

A large number of unit capsules can be prepared by filling standard two-piece hard gelatin capsules each
20 with 1000 mg of powdered active ingredient, 150 mg of lactose, 50 mg of cellulose, and 6 mg magnesium stearic.

Soft Gelatin Capsules

25 A mixture of active ingredient in a digestible oil such as soybean oil, cottonseed oil or olive oil can be prepared and injected by means of a positive displacement pump into gelatin to form soft gelatin capsules containing 1000 mg of the active ingredient. The
30 capsules should then be washed and dried.

Tablets

A large number of tablets can be prepared by conventional procedures so that the dosage unit is 1000 mg of active ingredient, 0.2 mg of colloidal silicon dioxide, 5 milligrams of magnesium stearate, 275 mg of microcrystalline cellulose, 11 mg of starch and 98.8 mg of lactose. Appropriate coatings may be applied to increase palatability or delay absorption.

Suspension

10

An aqueous suspension can be prepared for oral administration so that each 5 mL contain 25 mg of finely divided active ingredient, 200 mg of sodium carboxymethyl cellulose, 5 mg of sodium benzoate, 1.0 g of sorbitol solution, U.S.P., and 0.025 mg of vanillin.

Injectable

A parenteral composition suitable for administration by injection can be prepared by stirring 1.5% by weight of active ingredient in 10% by volume propylene glycol and water. The solution is sterilized by commonly used techniques.

Combination of components (a) and (b)

Each therapeutic agent component of this invention can independently be in any dosage form, such as those described above, and can also be administered in various ways, as described above. In the following description component (b) is to be understood to represent one or more agents as described previously. Thus, if components (a) and (b) are to be treated the same or independently,

each agent of component (b) may also be treated the same or independently. Components (a) and (b) of the present invention may be formulated together, in a single dosage unit (that is, combined together in one capsule, tablet, powder, or liquid, etc.) as a combination product. When component (a) and (b) are not formulated together in a single dosage unit, the component (a) may be administered at the same time as component (b) or in any order; for example component (a) of this invention may be administered first, followed by administration of component (b), or they may be administered in the reverse order. If component (b) contains more than one agent, e.g., one RT inhibitor and one protease inhibitor, these agents may be administered together or in any order. When not administered at the same time, preferably the administration of component (a) and (b) occurs less than about one hour apart. Preferably, the route of administration of component (a) and (b) is oral. The terms oral agent, oral inhibitor, oral compound, or the like, as used herein, denote compounds, which may be orally administered. Although it is preferable that component (a) and component (b) both be administered by the same route (that is, for example, both orally) or dosage form, if desired, they may each be administered by different routes (that is, for example, one component of the combination product may be administered orally, and another component may be administered intravenously) or dosage forms.

As is appreciated by a medical practitioner skilled in the art, the dosage of the combination therapy of the invention may vary depending upon various factors such as

the pharmacodynamic characteristics of the particular agent and its mode and route of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the kind of concurrent treatment, the frequency of treatment, and the effect desired, as described above. The proper dosage of components (a) and (b) of the present invention will be readily ascertainable by a medical practitioner skilled in the art, based upon the present disclosure. By way of general guidance, typically a daily dosage may be about 100 milligrams to about 1.5 grams of each component. If component (b) represents more than one compound, then typically a daily dosage may be about 100 milligrams to about 1.5 grams of each agent of component (b). By way of general guidance, when the compounds of component (a) and component (b) are administered in combination, the dosage amount of each component may be reduced by about 70-80% relative to the usual dosage of the component when it is administered alone as a single agent for the treatment of HIV infection, in view of the synergistic effect of the combination.

The combination products of this invention may be formulated such that, although the active ingredients are combined in a single dosage unit, the physical contact between the active ingredients is minimized. In order to minimize contact, for example, where the product is orally administered, one active ingredient may be enteric coated. By enteric coating one of the active ingredients, it is possible not only to minimize the contact between the combined active ingredients, but also, it is possible to control the release of one of

these components in the gastrointestinal tract such that one of these components is not released in the stomach but rather is released in the intestines. Another embodiment of this invention where oral administration is desired provides for a combination product wherein one of the active ingredients is coated with a sustained-release material which effects a sustained-release throughout the gastrointestinal tract and also serves to minimize physical contact between the combined active ingredients. Furthermore, the sustained-released component can be additionally enteric coated such that the release of this component occurs only in the intestine. Still another approach would involve the formulation of a combination product in which the one component is coated with a sustained and/or enteric release polymer, and the other component is also coated with a polymer such as a low-viscosity grade of hydroxypropyl methylcellulose or other appropriate materials as known in the art, in order to further separate the active components. The polymer coating serves to form an additional barrier to interaction with the other component. In each formulation wherein contact is prevented between components (a) and (b) via a coating or some other material, contact may also be prevented between the individual agents of component (b).

Dosage forms of the combination products of the present invention wherein one active ingredient is enteric coated can be in the form of tablets such that the enteric coated component and the other active ingredient are blended together and then compressed into a tablet or such that the enteric coated component is

compressed into one tablet layer and the other active ingredient is compressed into an additional layer.

Optionally, in order to further separate the two layers, one or more placebo layers may be present such that the

5 placebo layer is between the layers of active ingredients. In addition, dosage forms of the present invention can be in the form of capsules wherein one active ingredient is compressed into a tablet or in the form of a plurality of microtablets, particles, granules
10 or non-perils, which are then enteric coated. These enteric coated microtablets, particles, granules or non-perils are then placed into a capsule or compressed into a capsule along with a granulation of the other active ingredient.

15
These as well as other ways of minimizing contact between the components of combination products of the present invention, whether administered in a single dosage form or administered in separate forms but at the
20 same time or concurrently by the same manner, will be readily apparent to those skilled in the art, based on the present disclosure.

Pharmaceutical kits useful for the treatment of HIV
25 infection, which comprise a therapeutically effective amount of a pharmaceutical composition comprising a compound of component (a) and one or more compounds of component (b), in one or more sterile containers, are also within the ambit of the present invention.
30 Sterilization of the container may be carried out using conventional sterilization methodology well known to those skilled in the art. Component (a) and component

(b) may be in the same sterile container or in separate sterile containers. The sterile containers of materials may comprise separate containers, or one or more multi-part containers, as desired. Component (a) and component
5 (b), may be separate, or physically combined into a single dosage form or unit as described above. Such kits may further include, if desired, one or more of various conventional pharmaceutical kit components, such as for example, one or more pharmaceutically acceptable
10 carriers, additional vials for mixing the components, etc., as will be readily apparent to those skilled in the art. Instructions, either as inserts or as labels, indicating quantities of the components to be administered, guidelines for administration, and/or
15 guidelines for mixing the components, may also be included in the kit.

Obviously, numerous modifications and variations of the present invention are possible in light of the above
20 teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.

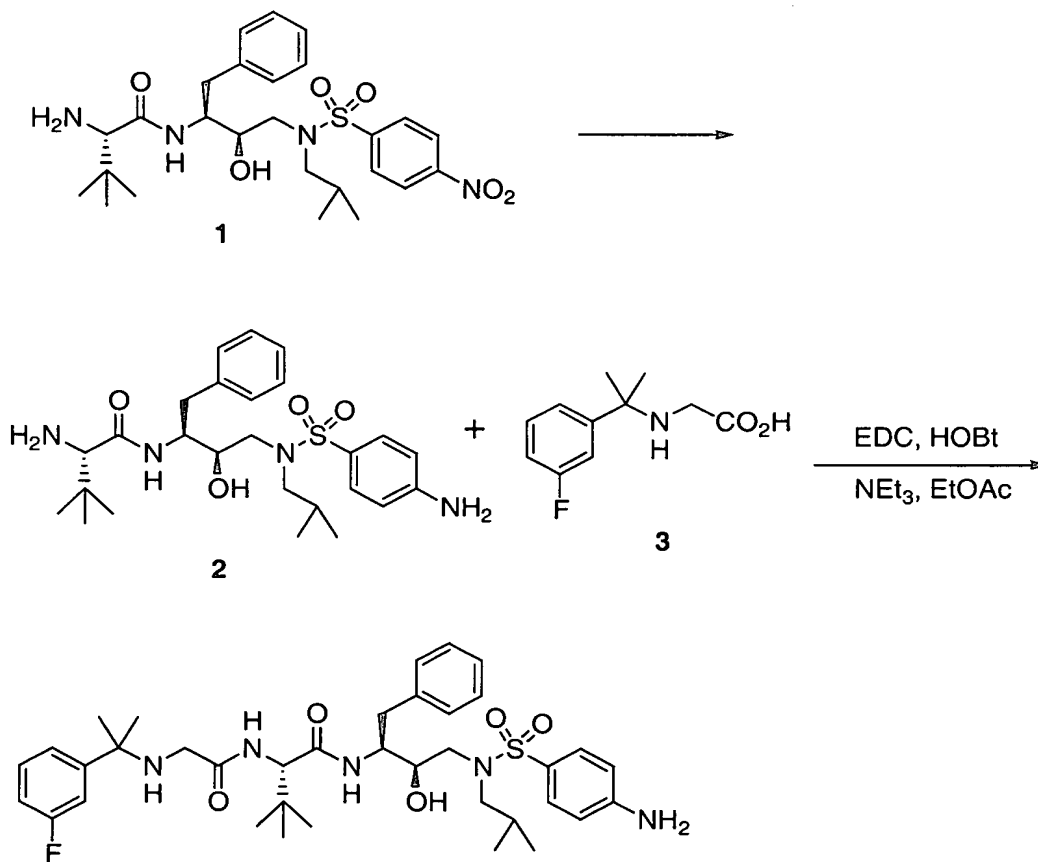
25 **SYNTHESIS**

Abbreviations used in the Examples are defined as follows: "atm" for atmosphere, "br s" for broad singlet, "°C" for degrees Celsius, "d" for doublet, , "eq" for
30 equivalent or equivalents, "g" for gram or grams, "mg" for milligram or milligrams, "mL" for milliliter or milliliters, "H" for hydrogen or hydrogens, "HPLC" for

high-pressure liquid chromatography, "m" for multiplet, "mmol" for millimole or millimoles, "hr" for hour or hours, "m" for multiplet, "M" for molar, "min" for minute or minutes, "MHz" for megahertz, "MS" for mass spectroscopy, "N" for normal, "nmr" or "NMR" for nuclear magnetic resonance spectroscopy, "s" for singlet, "t" for triplet, and "TLC" for thin layer chromatography.

Example 1

10

**Example 1**

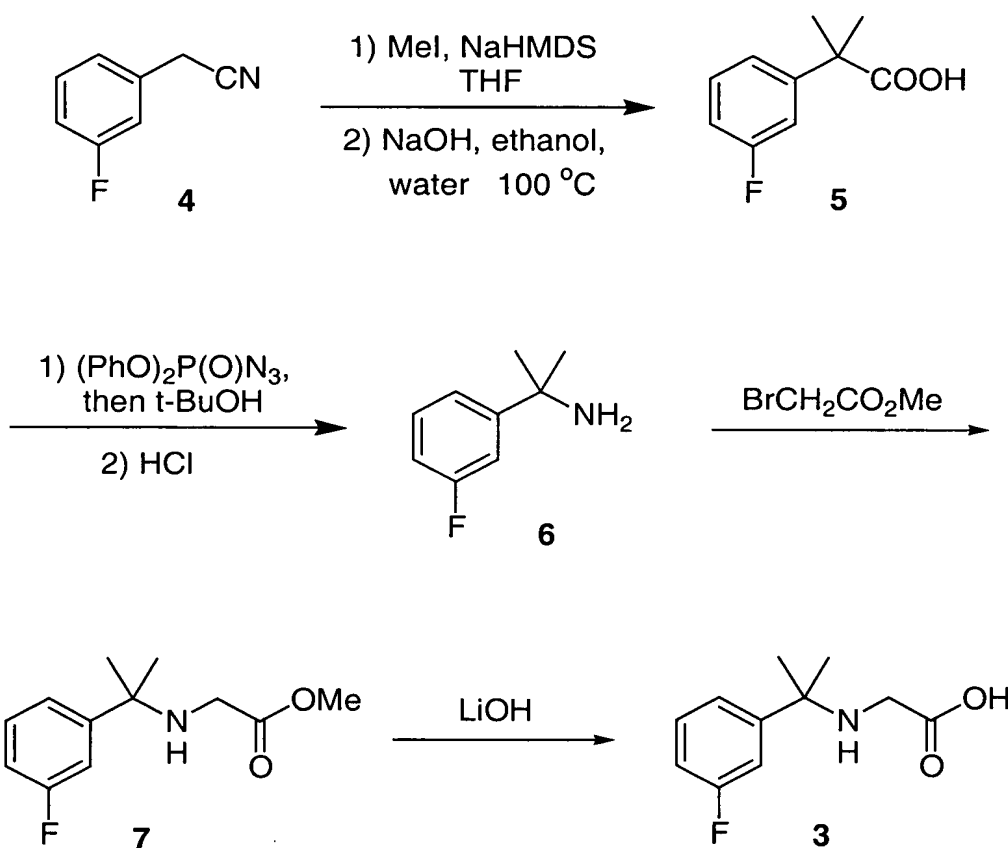
Step 1: A solution of 6.3 g (10 mmol) of **1** in water was made basic by the addition of 1 N aqueous sodium hydroxide solution, and the resulting mixture was

extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was dissolved in 50 mL of ethyl acetate and 50 mL of methanol. This solution was stirred at room temperature under hydrogen gas (1 atm.) in the presence of 0.60 g of 10% palladium on carbon until the reaction was complete as determined by reverse-phase HPLC. The mixture was filtered through celite, and the filtrate was concentrated under vacuum. The residue was dissolved in ethyl acetate, and an excess of 4 N hydrogen chloride in dioxane was added. The resulting precipitate was recovered by filtration and dried under vacuum to afford 4.5 g of **2**. Mass spec.: m/e 505 (M + H)⁺.

Step 2: To a mixture of 0.32 g (0.58 mmol) of **2**, 0.18 g (0.58 mmol) of N-[1-(3-fluorophenyl)-1-methylethyl]glycine(**3**), and 0.09 g (0.64 mmol) of 1-hydroxybenzotriazole in 10 mL of ethyl acetate at room temperature was added 0.33 mL (2.32 mmol) of triethylamine followed by 0.13 g (0.7 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. The resulting mixture was stirred at 40-45 °C for 4 hours. After cooling to room temperature the mixture was washed with water, 8% aqueous citric acid solution, water, 5% aqueous potassium carbonate solution, water, and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on silica gel (elution: 10-30% ethyl acetate/methylene) furnished Example 1 as its free-base. This material was dissolved in 5 mL of 1,4-dioxane to which was added an excess of 4 N hydrogen chloride in dioxane followed by 20 mL of

diethyl ether. The resulting precipitate was recovered by filtration, washed with diethyl ether, and dried under vacuum to furnish 0.24 g of Example 1 as its hydrochloride salt. High-resolution mass spec.: calc'd for $C_{37}H_{53}FN_5O_5S$ $(M+H)^+$: 698.3751; found: 698.3770.

Preparation of N-[1-(3-fluorophenyl)-1-methylethyl]glycine(3)



10

Step 1: To a solution of 7.2 g (53 mmol) of 3-fluorophenylacetonitrile(**4**) in 115 mL of tetrahydrofuran at 0 °C was added 118 mL of 1.00 M sodium hexamethyldisilazide in tetrahydrofuran, and this solution was stirred at 0 °C for 0.5 hour. To the solution was added 10.0 mL (161 mmol) of iodomethane, and

15

the reaction mixture was stirred at room temperature for 18 hours. The mixture was quenched at 0 °C by the addition of saturated aqueous ammonium chloride solution. The phases were separated, and the aqueous phase was
5 extracted with diethyl ether. The combined organic phases were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on silica gel (elution: 10% diethyl ether/hexane) afforded 8.0 g of 2-(3-fluorophenyl)-2-
10 methylpropionitrile. NMR(300 MHz, CDCl₃) δ 7.35 (d of d, 1 H), 7.25 (d of d of d, 1 H), 7.15 (d of t, 1 H), 6.99 (t of d, 1 H), 1.70 (s, 6H).

Step 2: A mixture of 6.2 g (38 mmol) of 2-(3-
15 fluorophenyl)-2-methylpropionitrile, 14 mL of ethanol, and 10 mL of 50% aqueous sodium hydroxide solution was stirred at 100 °C for 8 hours. After cooling the mixture was diluted with water and washed with ethyl acetate. The aqueous mixture then was adjusted to ~pH 2 with conc.
20 hydrochloric acid and extracted with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to provide 5.7 g of 2-(3-fluorophenyl)-2-methylpropionic acid(5). NMR(300 MHz, DMSO-d₆) δ 12.20 (br s, 1 H), 7.32
25 (d of d, 1 H), 7.13-6.97 (m, 3 H), 1.41 (s, 6H).

Step 3: To a solution of 5.74 g (36 mmol) of 2-(3-
fluorophenyl)-2-methylpropionic acid (5) in 180 mL of toluene at room temperature was added 5.5 mL (40 mmol) of
30 triethylamine followed by 7.8 mL (36 mmol) of diphenylphosphoryl azide. The resulting mixture was stirred at room temperature for 0.5 hour, heated slowly

to reflux over 0.5 hour, and then heated at reflux for 2 hours. The mixture was allowed to cooled to 40 °C, 20 mL of tert-butanol was added, and the resulting mixture was heated at reflux for another 20 hours. After cooling the
5 mixture was concentrated under vacuum, and the residue was dissolved in diethyl ether. This solution was washed with saturated aqueous sodium bicarbonate solution, water, and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on
10 silica gel (elution: 20% ethyl acetate/hexane) furnished 4.0 g of N-Boc-1-(3-fluorophenyl)-1-methylethylamine. NMR(300 MHz, CDCl₃) δ 7.27 (d of d, 1 H), 7.16 (d of d of d, 1 H), 7.09 (d of t, 1 H), 6.90 (t of d, 1 H), 4.95 (br s, 1 H), 1.60 (s, 6 H), 1.40 (s, 9 H).

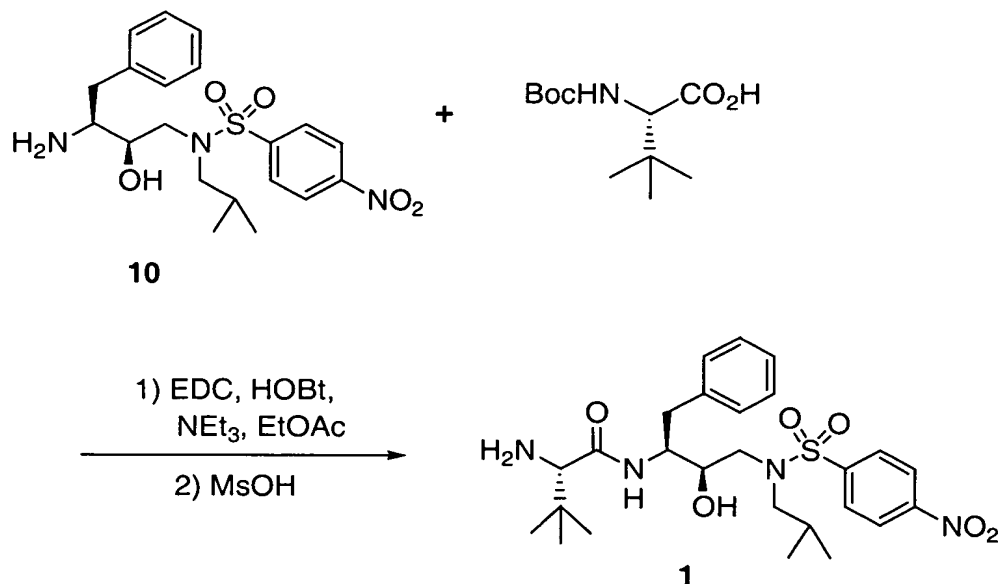
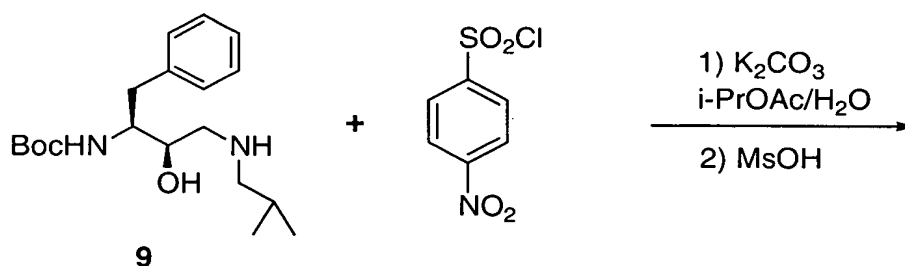
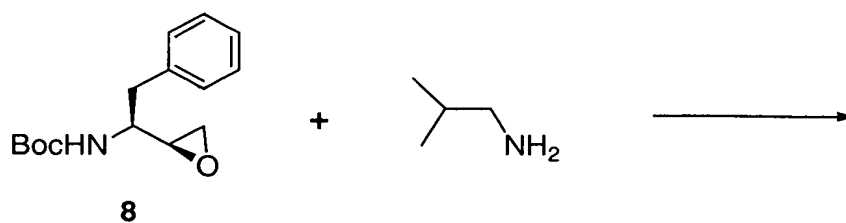
15

Step 4: To a solution of 4.0 g (15.8 mmol) of N-Boc-1-(3-fluorophenyl)-1-methylethylamine in 10 mL of ethyl acetate at room temperature was added 15 mL of 4 N hydrogen chloride in dioxane. The solution then was
20 stirred at room temperature for 2 hours. The solution then was concentrated under vacuum, and the residue was diluted with diethyl ether. The resulting suspension was filtered, and the solids were washed with diethyl ether and then dried to furnish 2.67 g of 1-(3-fluorophenyl)-1-methylethylamine(6) as its hydrochloride salt. NMR(300
25 MHz, CDCl₃) δ 9.02 (br s, 3 H), 7.40-7.29 (m, 3 H), 7.02 (t of d, 1 H), 1.81 (s, 6 H).

Step 5: To a mixture of 2.67 g (13.2 mmol) of 1-(3-fluorophenyl)-1-methylethylamine hydrochloride(6) and 4.0
30 g (30 mmol) of potassium carbonate in 40 mL of acetonitrile at room temperature was added slowly 1.25 mL

(13 mmol) of methyl bromoacetate. The reaction mixture was stirred at room temperature for 18 hours and then filtered. The filtrate was concentrated under vacuum, and the residue was dissolve in ethyl acetate. This
5 solution was washed with saturated aqueous sodium bicarbonate solution, water, and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on silica gel (elution: 20% ethyl acetate/hexane) provided 1.8 g of N-[1-(3-fluorophenyl)-
10 1-methylethyl]glycine, methyl ester(**7**). NMR (300 MHz, CDCl₃): δ 7.29 (d of d, 1 H), 7.21-7.11 (m, 2 H), 6.92 (t of d, 1 H), 3.69 (s, 3 H), 3.17 (s, 2 H), 1.46 (s, 6 H).

Step 6: To a solution of 1.8 g (8 mmol) of N-[1-(3-fluorophenyl)-1-methylethyl]glycine, methyl ester(**7**) in
15 40 mL of tetrahydrofuran at room temperature was added a solution of 0.67 g (16 mmol) of lithium hydroxide monhydrate in 10 mL of water. The reaction mixture was stirred at room temperature for 2 hours. The mixture was
20 concentrated under vacuum to ~5 mL of volume, adjusted to pH 2 with hydrochloric acid, and then diluted with brine. This mixture was extracted with 3:1 chloroform/isopropanol. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated
25 to afford 1.76 g of N-[1-(3-fluorophenyl)-1-methylethyl]glycine(**3**) as its hydrochloride salt. NMR(300 MHz, DMSO-d₆) δ 7.56-7.45 (m, 3 H), 7.27 (t of d, 1 H), 3.50 (s, 2 H), 1.72 (s, 6 H).

Preparation of Compound 1

5 Step 1: To a solution of 1000 mL of isobutylamine
 and 1000 mL of isopropanol at room temperature was added
 500 g of the commercially-available epoxide(**8**), and the
 resulting mixture was heated to reflux for 1 hour. After
 cooling, the mixture was concentrated under vacuum, and
 10 the residue was triturated with 1000 mL of heptane. The

resulting precipitate was recovered by filtration, washed with heptane, and dried to provide 625 g of the aminoalcohol(9).

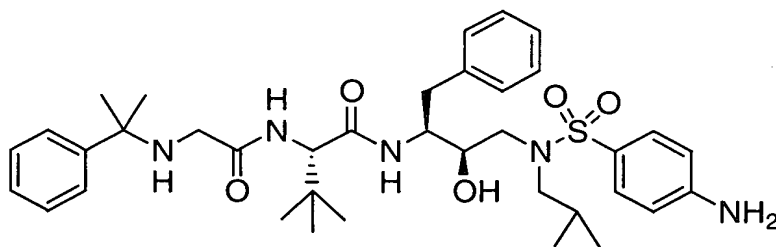
5 Step 2: To a solution of 100 g (300 mmol) of aminoalcohol(9) in 500 mL of isopropyl acetate was added a solution of 83 g (600 mmol) of potassium carbonate in 500 mL of water. The two-phase mixture was vigorously stirred and heated to 50 °C, and a solution of 69 g (312
10 mmol) of 4-nitrobenzenesulfonyl chloride in 200 mL of isopropyl acetate then was added, while the reaction mixture was maintained at 50 °C. Finally, the mixture was stirred at 50 °C for another 0.25 hour. After cooling, the phases were separated, and the organic phase
15 was washed with water and brine, dried over anhydrous sodium sulfate, and filtered. The resulting solution was heated to reflux (~85 °C), and 34.3 g (360 mmol) of methanesulfonic acid was added dropwise over 0.5 hour. The mixture was heated at reflux for another 0.5 hour and
20 then allowed to cool to room temperature. The resulting precipitate was recovered by filtration, washed with isopropyl acetate, and dried to afford 140 g of sulfonamide(10) as its methanesulfonate salt. Mass spec.: m/e 422 (M+H)⁺.

25

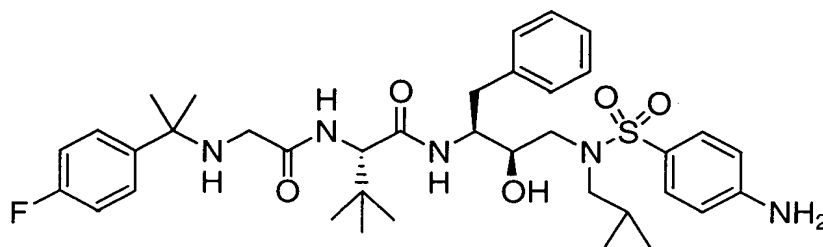
 Step 3: To a suspension of 60 g (116 mmol) of the product of step 2, 29.4 g (128 mmol) of N-Boc-L-tert-leucine, and 19.8 g (150 mmol) of 1-hydroxybenzotriazole in 540 mL of ethyl acetate at room temperature was added
30 74 mL (382 mmol) of triethylamine followed by 26.4 g (139 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. The resulting mixture was stirred at

40 °C for 2.5 hours. After cooling to room temperature the mixture was diluted with ethyl acetate. This solution was washed with 5% aqueous potassium carbonate solution, water, 8% aqueous citric acid solution, water, and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was dissolved in 900 mL of ethyl acetate. To this solution was added 7.40 g (150 mmol) of methanesulfonic acid, and the mixture was heated at reflux for 4 hours. After cooling, the suspension was filtered, and the recovered solids were washed with ethyl acetate and dried to furnish 65 g of **1** as its methanesulfonate salt. Mass spec.: m/e 535 ($M+H$)⁺.

Example 2

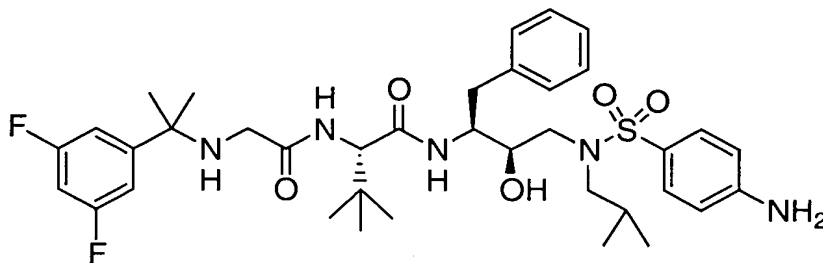


This compound was prepared as described for Example 1 employing α,α -dimethylbenzylamine as starting material. High-resolution mass spec.: calc'd for $C_{37}H_{54}N_5O_5S$ ($M+H$)⁺: 680.3846; found: 680.3843.

Example 3

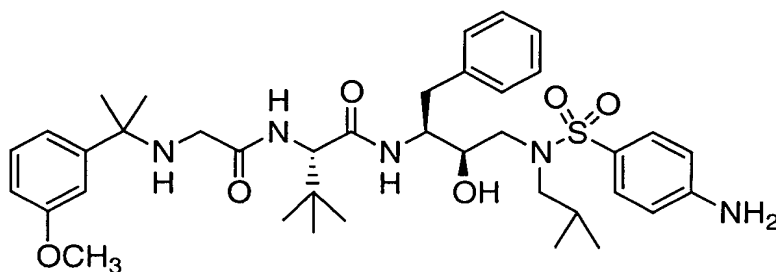
5 This compound was prepared as described for Example
1 employing 4-fluorophenylacetonitrile as starting
material. High-resolution mass spec.: calc'd for
 $C_{37}H_{53}FN_5O_5S$ $(M+H)^+$: 698.3751; found: 698.3762.

10

Example 4

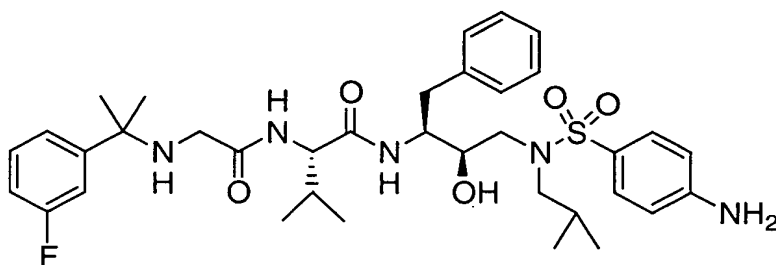
15 This compound was prepared as described for Example
1 employing 3,5-difluorophenylacetonitrile as starting
material. High-resolution mass spec.: calc'd for
 $C_{37}H_{52}F_2N_5O_5S$ $(M+H)^+$: 716.3657; found: 716.3681.

20

Example 5

5 This compound was prepared as described for Example 1 employing 3-methoxyphenylacetonitrile as starting material. High-resolution mass spec.: calc'd for $C_{38}H_{56}N_5O_6S$ $(M+H)^+$: 710.3951; found: 710.3933.

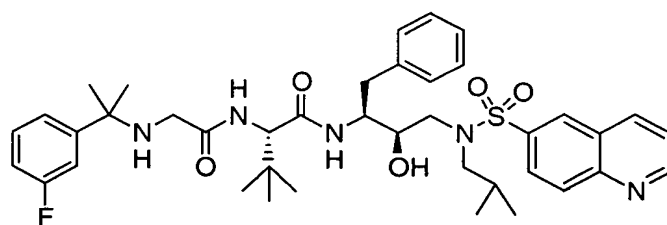
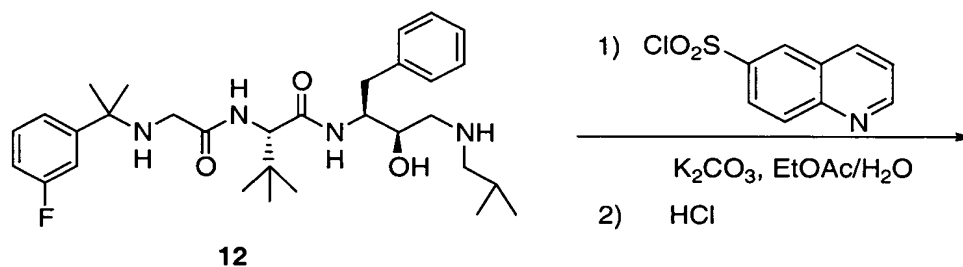
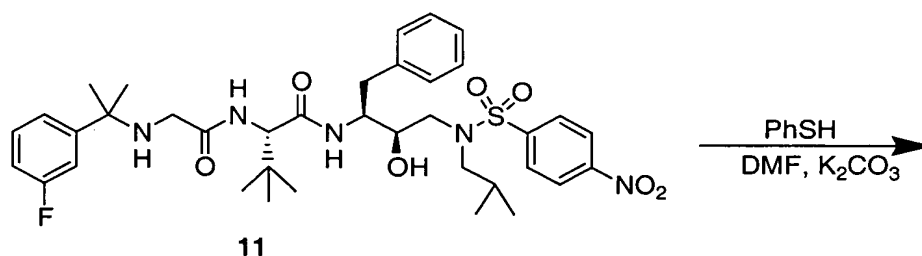
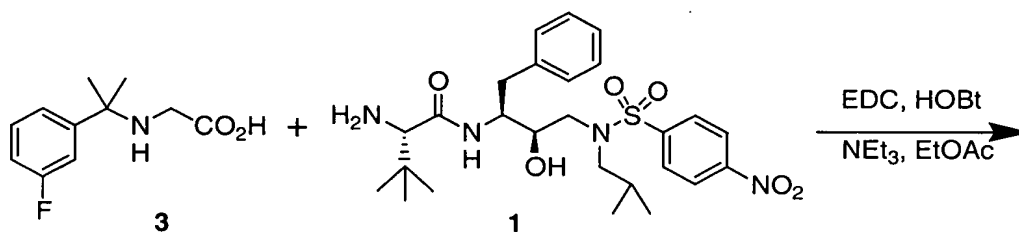
10

Example 6

15 This compound was prepared as described for Example 1 employing N-Boc-valine as starting material. High-resolution mass spec.: calc'd for $C_{36}H_{51}FN_5O_5S$ $(M+H)^+$: 684.3595; found: 684.3586.

20

57

Example 7

Example 7

5

Part 1: To a suspension of 3.2 g (5.0 mmol) of **1**, 1.17 g (5.5 mmol) of N-[1-(3-fluorophenyl)-1-methylethyl]glycine(**3**) from Example 1, and 0.75 g (5.5 mmol) of 1-hydroxybenzotriazole in 50 mL of ethyl acetate at room temperature was added 2.30 mL (16.5 mmol) of triethylamine followed by 1.2 g (5.5 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

The resulting mixture was stirred at 40 °C for 2.5 hours. After cooling to room temperature the mixture was diluted with ethyl acetate. This solution was washed with 5% aqueous potassium carbonate solution, water, 8% aqueous
5 citric acid solution, water, and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on silica gel (elution: 10-50% ethyl acetate/methylene chloride) provided 3.5 g of **11**. Mass spec.: m/e 728 (M+H)⁺.

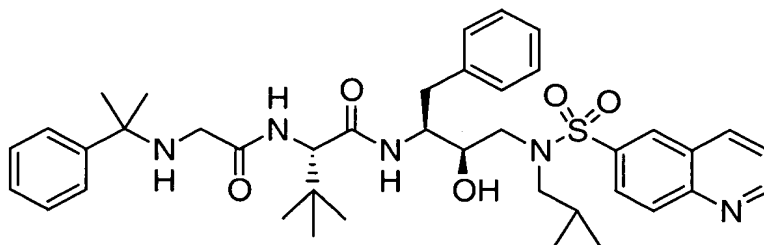
10

Step 2: To a mixture of 3.5 g (4.8 mmol) of **11** and 2.0 g (14.5 mmol) of potassium carbonate in dimethylformamide at room temperature was added 0.77 mL (7.5 mmol) of thiophenol, and the reaction mixture then
15 was stirred at room temperature for 2.5 hours. The mixture was poured into ethyl acetate, and the resulting solution was washed repeatedly with water and then brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on silica gel
20 (elution: 10% methanol/methylene chloride) furnished 2.0 g of **12**. Mass spec.: m/e 543 (M+H)⁺.

Step 3: A mixture of 0.22 g (0.4 mmol) of **12**, 0.14 g (0.6 mmol) of quinoline-6-sulfonyl chloride, and 0.55 g
25 (4.0 mmol) of potassium carbonate in 4 mL of ethyl acetate and 4 mL of water was stirred at room temperature for 16 hours. The mixture then was diluted with ethyl acetate, and the resulting solution was washed twice with water and then brine, dried over anhydrous sodium
30 sulfate, filtered, and concentrated to provide Example 7 as its free-base. To a solution of this material in 10 mL of ethyl acetate at room temperature was added an

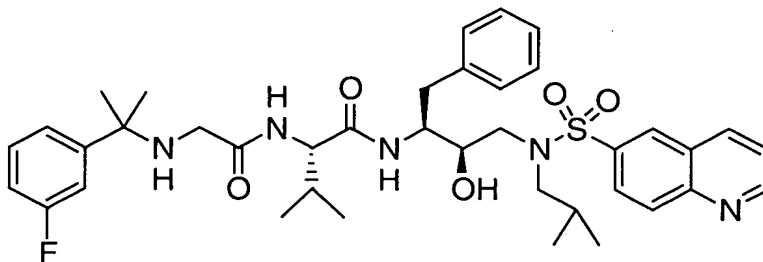
excess of 4 N hydrogen chloride in dioxane. The resulting mixture was diluted by slowly adding 40 mL of diethyl ether. The resulting precipitate was recovered by filtration, washed with diethyl ether, and dried under vacuum to afford 0.2 g of Example 7 as its hydrochloride salt. High-resolution mass spec.: calc'd for $C_{40}H_{53}FN_5O_5S$ ($M+H$)⁺: 734.3751; found: 734.3770.

Example 8



This compound was prepared as described for Examples 1 and 7 employing α,α -dimethylbenzylamine as starting material. High-resolution mass spec.: calc'd for $C_{40}H_{54}N_5O_5S$ ($M+H$)⁺: 716.3846; found: 716.3840.

Example 9

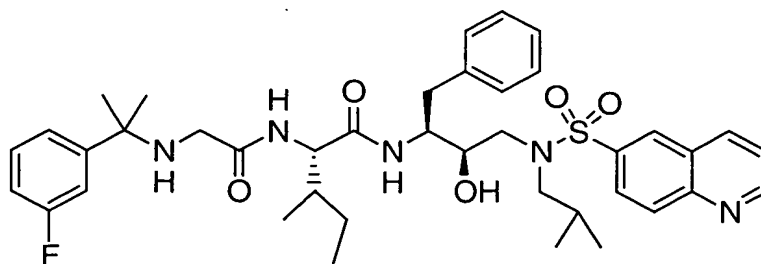


This compound was prepared as described for Examples 1 and 7 employing N-Boc-valine as starting material.

High-resolution mass spec.: calc'd for $C_{39}H_{51}FN_5O_5S$
(M+H)⁺: 720.3595; found: 720.3603.

Example 10

5

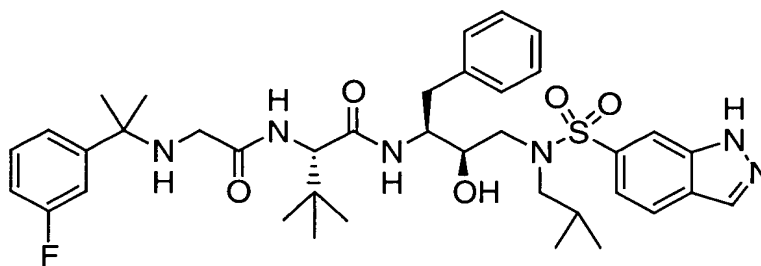


This compound was prepared as described for Examples 1 and 7 employing N-Boc-isoleucine as starting material.

10 High-resolution mass spec.: calc'd for $C_{40}H_{53}FN_5O_5S$
(M+H)⁺: 734.3751; found: 734.3764.

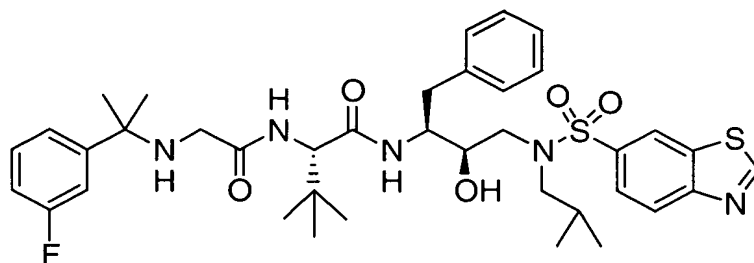
Example 11

15



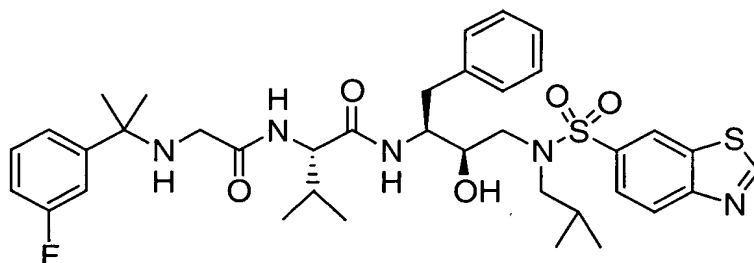
This compound was prepared as described for Example 7 employing indazole-6-sulfonyl chloride as starting material. High-resolution mass spec.: calc'd for

20 $C_{38}H_{52}FN_6O_5S$ (M+H)⁺: 723.3704; found: 723.3727.

Example 12

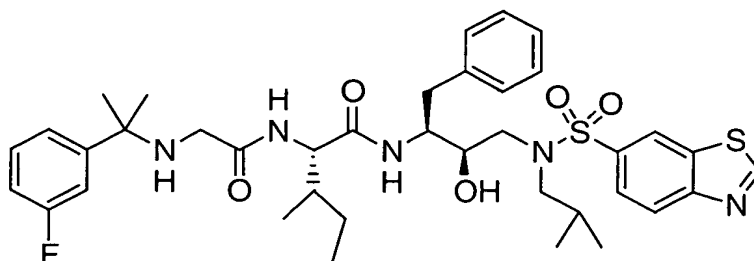
5 This compound was prepared as described for Example
7 employing benzothiazole-6-sulfonyl chloride as starting
material. High-resolution mass spec.: calc'd for
 $C_{38}H_{51}FN_5O_5S_2$ (M+H)⁺: 740.3316; found: 740.3331.

10

Example 13

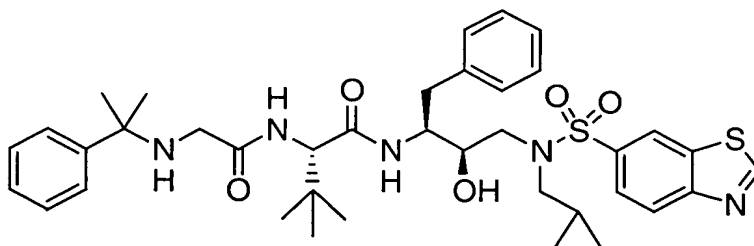
15 This compound was prepared as described for Examples
1 and 7 employing N-Boc-valine and benzothiazole-6-
sulfonyl chloride as starting materials. High-resolution
mass spec.: calc'd for $C_{37}H_{49}FN_5O_5S_2$ (M+H)⁺: 726.3159;
found: 726.3136.

20

Exempl 14

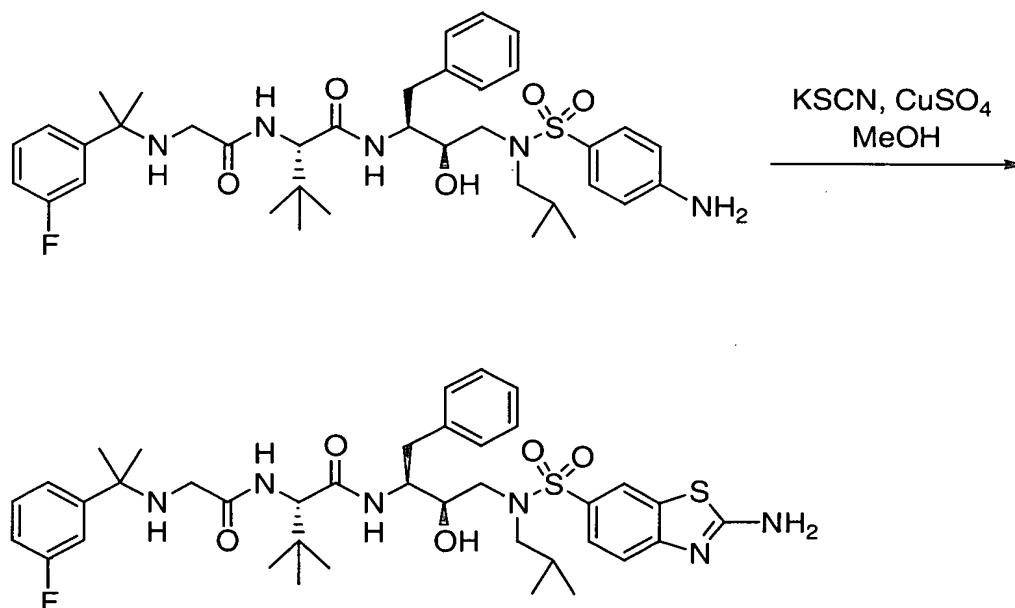
5 This compound was prepared as described for Examples
1 and 7 employing N-Boc-isoleucine and benzothiazole-6-
sulfonyl chloride as starting materials. High-resolution
mass spec.: calc'd for $C_{38}H_{51}FN_5O_5S_2$ $(M+H)^+$: 740.3316;
found: 740.3308.

10

Example 15

15 This compound was prepared as described for Examples
1 and 7 employing α,α -dimethylbenzylamine and
benzothiazole-6-sulfonyl chloride as starting materials.
High-resolution mass spec.: calc'd for $C_{38}H_{52}N_5O_5S_2$
 $(M+H)^+$: 722.3410; found: 722.3416.

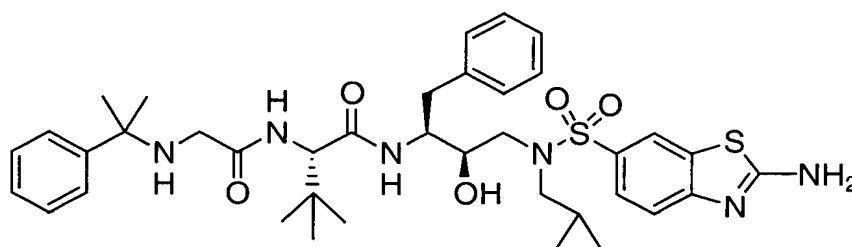
20

Examp1 16**Example 16**

5

A mixture of 0.30 g (0.4 mmol) of Example 1, 1.05 g of potassium thiocyanate, 0.84 g of copper(II)sulfate, and 5 mL of methanol was heated at reflux for 2 hours. After cooling, the mixture was filtered, diluted with 5 mL of water, and heated at reflux for another 1 hour. Finally, the mixture was diluted with 8 mL of ethanol, allowed to cool to room temperature, and filtered. The filtrate was concentrated under vacuum, and the residue was dissolved in ethyl acetate. The organic solution was washed twice with an aqueous ammonium chloride/ammonia buffer followed by brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on silica gel (elution: 2-5% methanol/methylene chloride) provided the pure product as its free-base. This material was dissolved in 5 mL of 1,4-dioxane to which was added excess of 4 N hydrogen

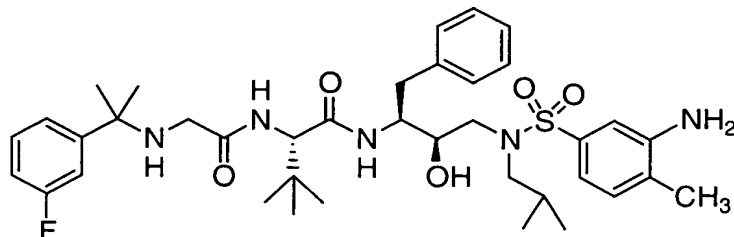
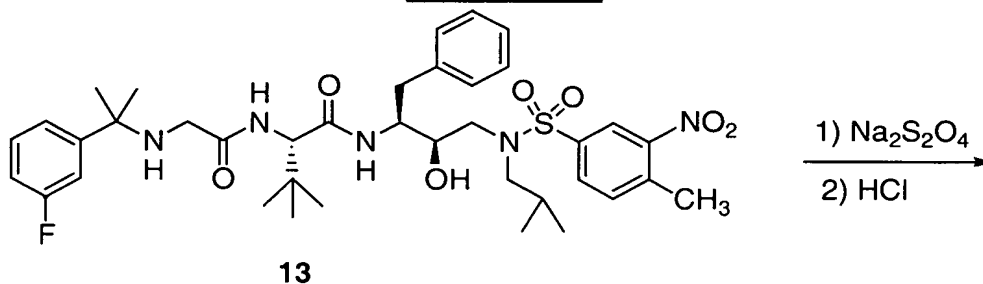
chloride in dioxane followed by ~20 mL of diethyl ether. The resulting precipitate was recovered by filtration, washed with diethyl ether, and dried under vacuum to afford Example 16 as its hydrochloride salt. High-resolution mass spec.: calc'd for $C_{38}H_{52}FN_6O_5S_2$ $(M+H)^+$: 755.3425; found: 755.3426.

Example 17

10

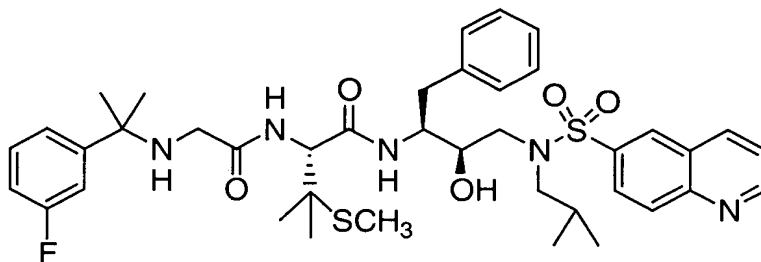
This product was prepared from Example 2 as described for Example 16. High-resolution mass spec.: calc'd for $C_{38}H_{53}N_6O_5S_2$ $(M+H)^+$: 737.3519; found: 737.3544.

15

Example 18**Example 18**

Step 1: To a solution of 0.37 g (0.5 mmol) of **13** (prepared as described for Example 7 employing 4-methyl-3-nitrobenzenesulfonyl chloride as starting material) in 10 mL of ethanol and 2 mL of conc. aqueous ammonium hydroxide at room temperature was added a solution of 0.70 g (4 mmol) of sodium dithionite in 4 mL of water, and the reaction mixture then was stirred at room temperature for 18 hours. The resulting mixture was diluted with water and then extracted with ethyl acetate. The combined extracts were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on silica gel (elution: 20-50% ethyl acetate/methylene chloride) afforded 0.24 g of Example 18 as its free-base. To a solution of this material in 10 mL of ethyl acetate was added an excess of 4 N hydrogen chloride in dioxane. The resulting precipitate was recovered by filtration, washed with diethyl ether, and dried under vacuum to provide 0.14 g of Example 18 as its hydrochloride salt. High-resolution mass spec.: calc'd for $C_{38}H_{55}FN_5O_5S$ (M+H)⁺: 712.3908; found: 712.3918.

Example 19



25

This compound was prepared as described for Examples 1 and 7 employing N-Boc-S-methylpenicillamine and

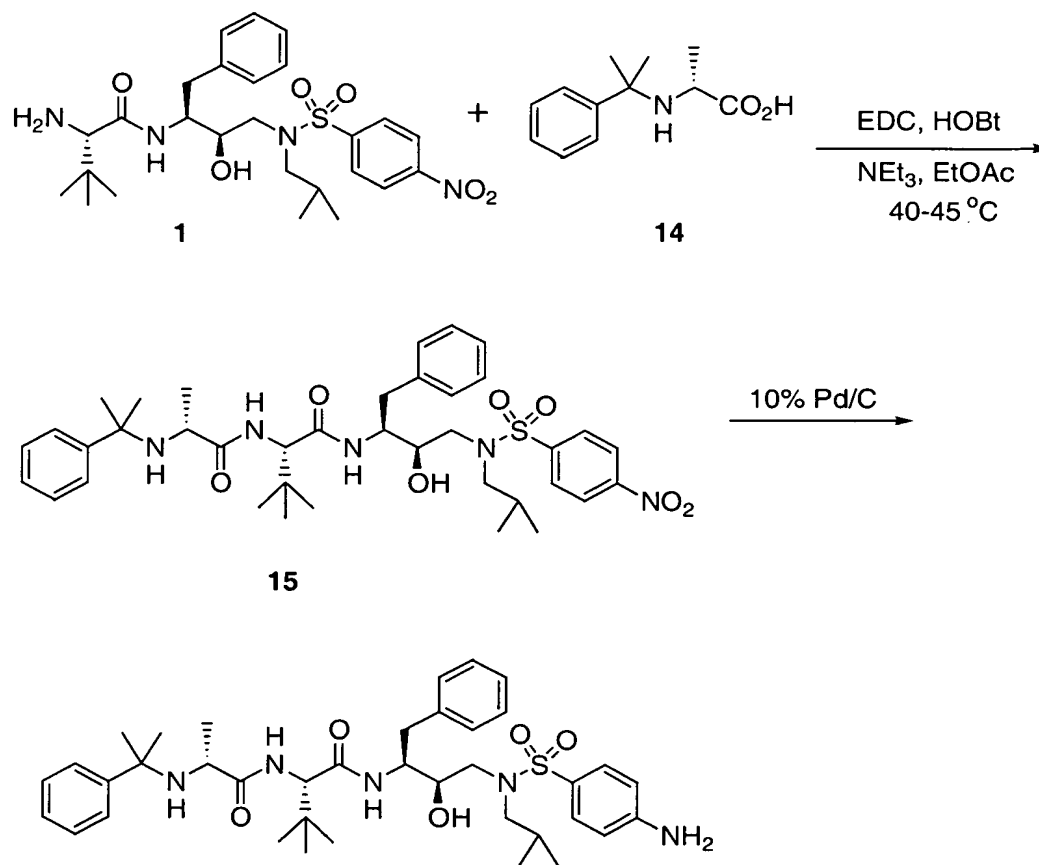
quinoline-6-sulfonyl chloride as starting materials.
High-resolution mass spec.: calc'd for $C_{40}H_{53}FN_5O_5S_2$
(M+H)⁺: 766.3472; found: 766.3476.

5 Preparation of N-Boc-S-methylpenicillamine

To a solution of 5.09 g (34.1 mmol) of penicillamine and 12 mL of 6 N aqueous sodium hydroxide solution in 75 mL of 1,4-dioxane and 25 mL of water at 0 °C was added
10 2.35 mL (37.6 mmol) of iodomethane. The reaction mixture was stirred at 0 °C for 3 hours followed by 2 hours at room temperature. The mixture was returned to 0 °C, and 8.7 g (40.0 mmol) of di-*tert*-butyl dicarbonate was added slowly. The resulting mixture was stirred at 0 °C for 1
15 hour followed by 14 hours at room temperature. The mixture then was concentrated under vacuum, and the residue was diluted with water. This aqueous phase was washed with diethyl ether, adjusted to ~pH 3 employing hydrochloric acid, and then extracted with ethyl acetate.
20 The combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to afford 7.0 g of the desired aminoacid.

25

30

Example 20**Example 20**

5

Step 1: To a mixture of 3.05 g (4.8 mmol) of **1**, 1.30 g (5.3 mmol) of N-(1-phenyl-1-methylethyl)-D-alanine hydrochloride(**14**), and 0.73 g (5.3 mmol) of 1-hydroxybenzotriazole in 48 mL of ethyl acetate at room temperature was added 2.70 mL (19.4 mmol) of triethylamine followed by 1.12 g (5.8 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. The resulting mixture was stirred at 40-45 °C for 4 hours. After cooling to room temperature the mixture was washed with water, 8% aqueous citric acid solution, water, 5% aqueous potassium carbonate solution, water,

and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on silica gel (elution: 20% ethyl acetate/methylene) furnished 3.10 g of **15**. Mass spec.: m/e 724 (M+H)⁺.

5

Step 2: A mixture of 0.28 g of **15**, 0.050 g of 10% palladium on carbon, and 10 mL of ethyl acetate was stirred at room temperature under hydrogen gas (1 atm.) for 18 hours. The resulting mixture was filtered through celite[®], and the filtrate was concentrated under vacuum. Column chromatography on silica gel (elution: 5% methanol/methylene chloride) afforded 0.20 g of Example 20 as its free-base. To this material in 3 mL of dioxane was added 1 mL of 4 N hydrogen chloride in dioxane followed by the dropwise addition of ~15 mL of diethyl ether. The resulting precipitate was recovered by filtration, washed with diethyl ether, and dried under vacuum to provide 0.20 g of Example 20 as its hydrochloride salt. High-resolution mass spec.: calc'd for C₃₈H₅₆N₅O₅S (M+H)⁺: 694.4002; found: 694.4017.

20

Preparation of N-(1-phenyl-1-methylethyl)-D-alanine hydrochloride(14)

Step 1: To a solution of 4.05 g (30 mmol) of α,α -dimethylbenzylamine in 30 mL of methylene chloride at room temperature was added 2.50 g (10 mmol) of ethyl O-trifluoromethanesulfonyl-L-lactate, and the reaction mixture was stirred at room temperature for 18 hours. The mixture was washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. Column chromatography on silica gel (elution: 0-5% ethyl

25

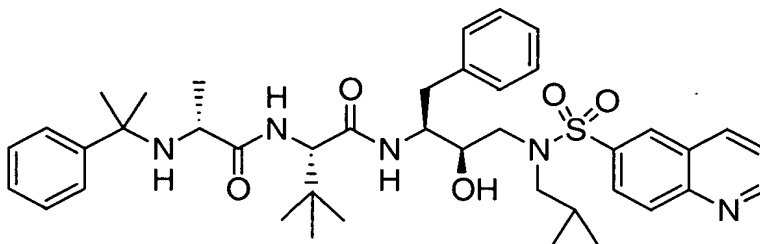
30

acetate/methylene chloride) furnished 2.22 g of N-(1-phenyl-1-methylethyl)-D-alanine, ethyl ester. NMR (300 MHz, CDCl₃) δ 7.46 (m, 2 H), 7.34-7.18 (m, 3 H), 4.04 (quart., 2 H), 3.06 (quart., 1 H), 1.44 (s, 3 H), 1.41 (s, 3 H), 1.22-1.17 (m, 6 H).

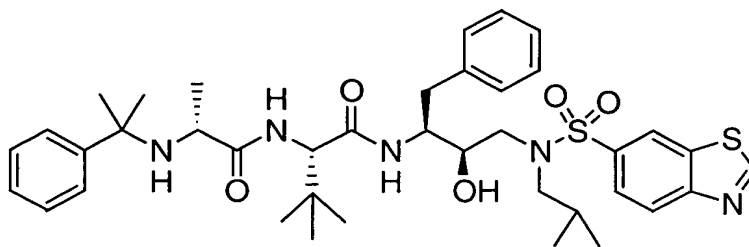
Step 2: To a solution of 2.22 g of N-(1-phenyl-1-methylethyl)-D-alanine, ethyl ester in 25 mL of ethanol at room temperature was added an excess of 50% aqueous sodium hydroxide solution, and the reaction mixture was stirred at room temperature for 4 hour. The mixture was concentrated under vacuum, and the residue was diluted with brine. The aqueous mixture was adjusted to pH 2-3 with conc. hydrochloric acid and then was extracted with 1:4 isopropanol/chloroform. The combined extracts were dried over anhydrous sodium sulfate, filtered, and concentrated to afford 1.40 g of N-(1-phenyl-1-methylethyl)-D-alanine hydrochloride(**14**).

20

Example 21

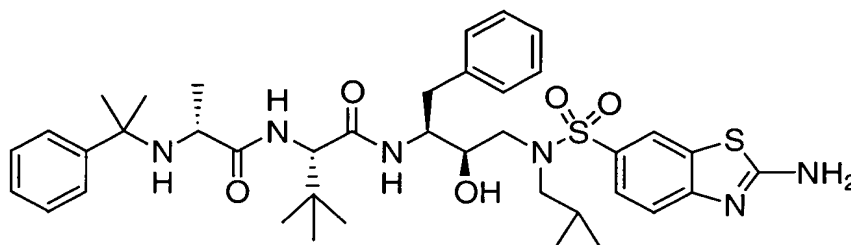


This compound was prepared as described for Example 7 employing **15** from Example 20 and quinoline-6-sulfonyl chloride as starting materials. High-resolution mass spec.: calc'd for C₄₁H₅₆N₅O₅S (M+H)⁺: 730.4002; found: 730.4016.

Example 22

5 This compound was prepared as described for Example
7 employing **15** from Example 20 and benzothiazole-6-
sulfonyl chloride as starting materials. High-resolution
mass spec.: calc'd for $C_{39}H_{54}N_5O_5S_2$ $(M+H)^+$: 736.3566;
found: 736.3582.

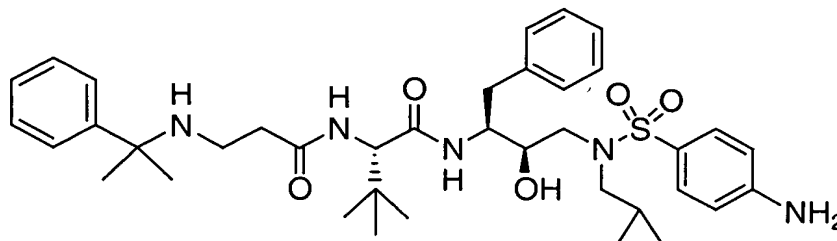
10

Example 23

15 This product was prepared from Example 20 as
described for Example 17. High-resolution mass spec.:
calc'd for $C_{39}H_{55}N_6O_5S_2$ $(M+H)^+$: 751.3675; found: 751.3662.

Example 24

20



This compound was prepared as described for Example 1 employing N-(1-phenyl-1-methylethyl)- β -alanine as starting material. High-resolution mass spec.: calc'd for $C_{38}H_{56}N_5O_5S$ (M+H)⁺: 694.4002; found: 694.4005.

5

Preparation of N-(1-phenyl-1-methylethyl)- β -alanine

Step 1: A solution of 0.73 g (5.4 mmol) of α,α -dimethylbenzylamine, 0.59 g (5.4 mmol) of methyl 3-bromopropionate, and 1.49 g (10.8 mmol) of potassium carbonate in 15 mL of acetonitrile and 2.5 mL of water was stirred at room temperature for 48 hours. The mixture was diluted with water and then extracted with ethyl acetate. The combined extracts were washed with water and brine, dried over anhydrous magnesium sulfate, filtered, and concentrated. Column chromatography on silica gel (elution: 5% methanol/methylene chloride) furnished 0.23 g of N-(1-phenyl-1-methylethyl)- β -alanine, methyl ester. NMR (300 MHz, $CDCl_3$) δ 7.44 (d, 2 H), 7.33 (t, 2 H), 7.21 (t, 1 H), 3.67 (s, 3 H), 2.58 (t, 2 H), 2.44 (t, 2 H), 1.46 (s, 6 H).

Part 2: A mixture of 0.22 g (1.0 mmol) of N-(1-phenyl-1-methylethyl)- β -alanine, methyl ester, 3 mL of 1.0 N lithium hydroxide in water, and 3 mL of methanol was stirred at room temperature for 3.5 hours. The resulting mixture was concentrated to dryness under vacuum, and the residue was suspended in brine. This mixture was extracted with 9:1 methylene chloride/methanol, and the combined extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated to furnish 0.060 g of N-(1-phenyl-1-

methylethyl)- β -alanine. NMR(300 MHz, CD₃OD) δ 7.59-7.40 (m, 5 H), 2.93 (t, 2 H), 2.60 (t, 2 H), 1.80 (s, 6 H).